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# A Closer Look at Brazilwood and its Lake Pigments

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# A Closer Look at Brazilwood and its Lake Pigments

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**Abstract**

Brazilwood is a soluble redwood that was used as source of organic dye and for lake pigments. It was widely applied in medieval times to colour textiles and in the form of pigment for manuscript illumination and, although it is relatively unstable, it is still found in the 19$^{th}$ oil palette. However, this colorant has received little attention. In this sense, the present work aims to contribute knowledge on the characterisation of brazilwood and its lake pigments.

The flavonoid brazilein is the red chromophore that can be extracted from the brazilwood tree. To study with more detail its behaviour, the molecule was isolated from brazilwood scrapes. As confirmed by NMR and HPLC-DAD, a high purity compound was obtained (for which a p$K_{a1}$ of 7 was determined) and a pure brazilein-$Al^{3+}$ complex was produced. Characterisation with UV-Vis spectroscopy and µ-spectrofluorimetry revealed that in its acidic form, the chromophore presents absorption, excitation and emission maxima at 446, 475 and 536 nm, respectively, and at 540, 548 and 560 nm when deprotonated. When complexed with $Al^{3+}$ these values are changed to 510, 520 and 585 nm.

Brazilwood lake reconstructions with as much historical accuracy as possible were also prepared based on recipes from the 15$^{th}$ century documentary text *Livro de como se fazem as cores* and the Winsor & Newton 19$^{th}$ century archive. This led to a greater understanding of the manufacture of the lakes and its evolution through time. Furthermore, in order to investigate the best strategy to characterise such pigments and paints, and to provide reference samples, the lakes produced and applied as temperas were then analysed by colorimetry, FTIR, SEM-EDS, FORS and UV-Vis µ-spectrofluorimetry.

Despite some differences exist between the two sources, mainly with respect to the dye extraction method, both are guided by the same principles and are *constructed* in order that the same final pH, adequate for pigment precipitation, is obtained. In particular, the 15$^{th}$ century *Livro de como se fazem as cores*, so distant in time, allowed for the successful preparation of brazilwood lake pigments, which can be obtained in a variety of different hues from light pink to dark red and purple. Concerning the analysis carried out, FTIR was essential to characterise extenders, while FORS spectra ($\lambda_{abs}$ 560 nm) and µ-spectrofluorimetry ($\lambda_{ex}$ 553 nm for excitation and $\lambda_{em}$ 585-90 nm for emission) data were very consistent and fundamental to identify the brazilwood chromophore. These three techniques were essential and complementary in the characterisation of the paints produced.

Medieval reconstructions were compared with pink and red colours of illuminations found in three French books of hours (*Palácio Nacional de Mafra*, Portugal) from the 15$^{th}$ century. Good correlation between FTIR, FORS and µ-spectrofluorimetry spectra of the reconstructions and historic samples suggest that the illuminations colours were prepared with brazilwood lakes.

*Keywords:* book of hour, brazilein, brazilwood, lake pigment characterisation, *Livro de como se fazem as cores*, Winsor & Newton

**Resumo**

Pau-brasil é uma madeira vermelha solúvel que foi usada enquanto fonte de corante orgânico e para produzir pigmentos laca. Foi largamente utilizado na época medieval para colorir têxteis e na forma de pigmento para a iluminação de manuscritos e, apesar de ser relativamente instável, é ainda encontrado na paleta a óleo do século XIX. Porém, este colorante têm recebido pouca atenção. Neste sentido, o presente trabalho procura contribuir com um maior conhecimento no que diz respeito à caracterização do pau-brasil e dos seus pigmentos laca.

O flavonoide brasileína é o cromóforo vermelho que pode ser extraído da árvore do pau-brasil. Para estudar com mais detalhe o seu comportamento, a molécula foi isolada a partir de raspas de pau-brasil. Tal como confirmado por RMN e HPLC-DAD, obteve-se um composto puro (para o qual se determinou um $pK_{a1} = 7$), a partir do qual se produziu o complexo brasileína-$Al^{3+}$. A caracterização por espectroscopia UV-Vis e µ-espectrofluorimetria revelou que, na forma ácida, o cromóforo apresenta máximos de absorção, excitação e emissão a 446, 475 e 536 nm, respectivamente, e a 540, 548 e 560 nm quando desprotonado. Quando complexado com $Al^{3+}$, estes valores são alterados para 510, 520 e 585 nm.

Por outro lado, foram preparadas reconstruções de pigmentos laca de pau-brasil com o maior rigor histórico possível, com base em receitas da fonte documental do século XV *Livro de como se fazem as cores* e do arquivo do século XIX da Winsor & Newton. Esta preparação levou a uma maior compreensão acerca da manufactura das lacas e da sua evolução ao longo do tempo. Para além disso, de modo a investigar a melhor estratégia para caracterizar tais pigmentos e tintas, e de maneira a criar amostras de referência, as lacas produzidas e aplicadas na forma de têmpera foram analisadas por colorimetria, FTIR, SEM-EDS, FORS e µ-espectrofluorimetria UV-Vis.

Apesar de existirem algumas diferenças entre as duas fontes documentais, principalmente no que diz respeito ao método de extracção do corante, ambas são guiadas pelos mesmos princípios e construídas de forma a que o mesmo pH final, adequado para a precipitação do pigmento, seja obtido. Em particular, o *Livro de como se fazem as cores* do século XV, tão distante no tempo, permitiu uma boa preparação de pigmentos laca de pau-brasil, os quais podem ser obtidos numa variedade de diferentes tons desde rosa claro a vermelho escuro ou roxo. No que diz respeito às análises realizadas, o FTIR foi essencial para caracterizar as cargas presentes nos pigmentos, enquanto que os resultados do FORS ($\lambda_{abs}$ 560 nm) e µ-espectrofluorimetria ($\lambda_{ex}$ 553 nm, excitação; $\lambda_{em}$ 585-90 nm, emissão) foram bastante consistentes e fundamentais para identificar o cromóforo de pau-brasil. Estas três técnicas foram essenciais e complementares entre si na caracterização das tintas produzidas.

Por fim, as reconstruções medievais foram comparadas com as cores rosas e vermelhas de iluminuras encontradas em três livros de horas franceses (Palácio Nacional de Mafra, Portugal) do século XV. Uma boa correspondência entre os espectros de FTIR, FORS e µ-espectrofluorimetria das reconstruções e das amostras históricas sugere que as cores das iluminuras foram preparadas com lacas de pau-brasil.

*Termos chave:* livro de horas, brasileína, pau-brasil, caracterização de pigmentos laca, *Livro de como se fazem as cores*, Winsor & Newton

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| | |
|---|---|
| **NMR** | Nuclear Magnetic Resonance Spectroscopy |
| **HPLC-DAD** | High Performance Liquid Chromatography equipped with Diode Array Detector |
| **UV-Vis** | Ultraviolet-Visible Spectroscopy |
| **FTIR** | Fourier Transform Infrared Spectroscopy |
| **FORS** | UV-Visible-NIR Fibre Optic Reflectance Spectroscopy |
| **SEM-EDS** | Scanning Electron Microscopy equipped with Energy Dispersive Spectroscopy |
| **W&N** | Winsor & Newton |
| **PNM** | *Palácio Nacional de Mafra* |
| **Ms** | Manuscript |
| **f** | Folio |
| **DCR** | Department of Conservation and Restoration |
| **Log(1/R)** | Apparent absorbance |
| $\lambda_{max}$ | Absorption maximum |
| $\nu$ | Stretching vibration |
| $\delta$ | Bending vibration |
| $K_{sp}$ | Solubility Product Constant |
| $t_R$ | Retention time |
| $\eta$ | Yield |

## 1. Introduction

### 1.1. Preamble

The study of the materials and techniques used in the past to create our cultural heritage is of great value. It helps to understand and place in context the artistic works, to solve authenticity problems, and allows for more effective and adequate restoration and conservation procedures. Natural organic dyes[1] of both vegetable and animal origin and their complexes have been used for works of art such as illuminated manuscripts, paintings and textiles. In order to recognise these dyes in such artefacts, it is essential to characterise its molecular structure and behaviour. Moreover, because they are often found as dye-metal complexes, in a solid state together with extenders and binders, it is also fundamental to perform pigment and paint reconstructions with as much historical accuracy as possible. These are prepared following reliable and contemporary documentary sources of technical information [2]. They are then characterised with analytical techniques providing reference samples. This is important because modern materials do not represent those from the past and cannot be used to give insight into it [3].

It has been stated that brazilwood was the greatest source for red colours in the Middle Ages for painting and dyeing, and its presence has been identified in illuminations of 15$^{th}$ century French manuscripts [4, 5]. Also, despite its fugitive character, brazilwood was still used in the 19$^{th}$ century for oil painting. However, unlike other organic reds (e.g. madder and cochineal), this soluble redwood has received little attention. As such, this work aims to contribute to a better knowledge of this colorant.

To study the brazilwood chromophore[2], brazilein was isolated and Nuclear Magnetic Resonance spectroscopy (NMR) and High Performance Liquid Chromatography equipped with Diode Array Detector (HPLC-DAD) were used to confirm its molecular structure and purity. Brazilein behaviour was then studied both with Ultraviolet-Visible spectroscopy (UV-Vis) and spectrofluorimetry, a promising technique for the characterisation of chromophores based on their fluorescence [6, 7].

To better understand the manufacture of brazilwood lakes and their evolution since medieval to more recent times, historically accurate 15$^{th}$ and 19$^{th}$ reconstructions were prepared from two distinct sources. Medieval recipes were selected from the 15$^{th}$ century *Livro de como se fazem as cores*, the only Portuguese documentary source on the art of manuscript illumination. The 19$^{th}$ century archive of the internationally renowned colourman Winsor & Newton (W&N) that constitutes an important source of information for artists' materials, was the second source for brazilwood lakes production.

Lake pigments and paints are composed by the coloured dye, the substrate and the binder. To provide their complete characterisation, a multi-analytical approach was used. The lakes were characterised by Fourier Transform Infrared spectroscopy (FTIR), a fingerprinting technique that gives information at the molecular level [8]. Then, analysis with UV-Visible-NIR Fibre Optic Reflectance Spectroscopy (FORS) and UV-Vis microspectrofluorimetry, two valuable techniques in the identification of dyes, was carried out [9, 10]. These two techniques can be used *in situ* allowing to collect information in a non-invasive away, which consists of an advantage when works of art are concerned. Paints colour coordinates, expressed in the L*a*b* colour space, were also acquired [11]. Information on the morphology and elements of selected

[1] Dyes and pigments can be distinguished based on their solubility in the media. While the former are generally soluble organic compounds, the latter are usually inorganic compounds insoluble and dispersed in the medium. [1]

[2] Chromophores are molecules that contain unsaturated functional groups, capable of absorbing UV-visible radiation therefore bringing or creating colour [1, 6].

paints was also obtained by Scanning Electron Microscopy equipped with Energy Dispersive Spectroscopy (SEM-EDS) [12]. Results were compared with data obtained for brazilein and brazilein lakes and for case-study colours from French books of hours (15th century) from *Palácio Nacional de Mafra*.

### 1.2. Brazilwood and its Lake Pigments

Brazilwood was used as natural source for red colours in ancient Asian civilizations [13, 14]. In the early Middle Ages it started to be imported on a large scale from Sri Lanka, India and Southeast Asia to Europe [1, 13, 14]. It was by then that this soluble redwood, known as brazil, *bresil*, *brasilium*, *verzi* or *verzino* (among other variations), became widely applied, mainly as source of dye for inks and to colour textiles, but also to prepare lake pigments (dye-metal complexes) for manuscript illumination [5, 15, 16]. Believed to have provided the foremost red colour of medieval times, brazilwood was among the colouring materials widely available in European markets [4, 14, 15].

At this time, the Asian sappanwood, *Caesalpinia sappan*, was the only brazilwood species known as such [13]. However, the discovery of South America in the beginning of the 16th century by the Portuguese meant the discovery of a new world of trees similar to sappanwood with dyeing properties as well[3] [14, 17]. These started to be imported to Europe replacing that from the Orient. Yet, not only the new redwoods belonged to different species, but they also started to be exploited in different regions of Central and South America. As a consequence, many of them were named either after their place of origin or the harbour from where they were shipped without any relation to the botanical species. This resulted in a variety of names such as brazil, pernambuco, lima and nicaragua wood, which makes it difficult to identify which species were meant. Despite sometimes particular species could be mentioned, brazilwood became a general term to designate all soluble redwoods used as dyestuff. [13, 14]

Information found in the literature with respect to redwood species, their names and places of origin is somewhat contradictory and often the same name can correspond to different species and vice versa [18]. In summary the main brazilwood species from both Asia and America are: *Caesalpinia sappan*, *Caesalpinia echinata*, *Caesalpinia brasiliensis*, *Caesalpinia violacea*, *Caesalpinia crista* and *Haematoxylum brasiletto* [1, 13, 18, 19]. All these redwoods belong to the Fabales order, Leguminosae family [14]. Some of them are still important today, not so much as sources of red dyes, but for the medicinal properties of their components (*C. sappan*) or because their wood is considered the ideal material for making violin bows (*C. echinata*, which is now considered an endangered species) [13, 20].

For dyeing, only the coloured heartwood of the trunk and larger branches of the trees were used. In the Middle Ages, sappanwood was traded in the form of logs. Some medieval dyers then had the wood cut up into chips with billhooks and ground into powder in mortars. Later, brazilwoods from America were cut up and would go into commerce either as logs or rasped chips, which were then reduced to powder. By this time, prison inmates or mills built for the purpose were used to do so. [13, 22]

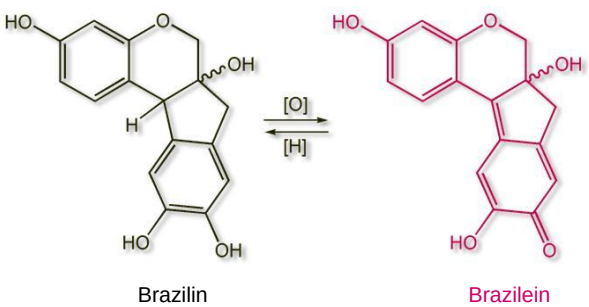


**Figure 1.** Brazilin (reduced form) and brazilein (oxidised form).

The almost colourless flavonoid brazilin ($C_{16}H_{14}O_5$) is the main source of colorant in the

[3] In fact, it is told that the country Brazil in South America was named as such after the discovery of these trees that looked like those from the Orient known as brazilwood [14].

heartwood of brazilwood species [13, 16]. Isolated in 1808, its heterocyclic structure (composed of two aromatic rings, one five-membered ring, a pyrone and four hydroxyl groups) was only fully established in 1833 (Figure 1) [13, 21]. It is the natural precursor of the oxidised form brazilein ($C_{16}H_{12}O_5$)[4], the real red chromophore with much stronger colouring power and which structure has been recently studied by Berger and Sicker (2009). It is stated that in contact with oxygen in the air and light brazilin is readily converted to brazilein due to the oxidation of one hydroxyl group to a carbonyl [14, 19]. Different brazilwood species contain brazilin and other phenolic compounds that are potential sources of colouring matter (including precursors of brazilin) but in different ratios [13, 22].

The term lake pigment could designate a pigment prepared either from brazilwood or from anthraquinones, the most stable and important natural red dyes, which include kermes, lac, cochineal and madder [1, 16, 23]. These pigments were traditionally prepared by extracting the dye from its natural plant or animal source[5] and precipitating it in solution with inorganic salts at neutral or weakly acidic pH [26]. These salts are generally called mordants. The latter binds to specific functional groups in the dye molecule forming a metal-dye complex and converting the water-soluble dye into an insoluble pigment [27]. Alum[6], a source for aluminium ions ($Al^{3+}$) was the most common mordant. In this case, amorphous hydrated alumina will be the product of the reaction between alum and an alkaline component. The dye will bind or/and adsorb to this colloidal substrate [16, 23, 25].

Brazilwood dye could be extracted in neutral or basic aqueous solutions to which alum was added. White substances such as chalk (calcium carbonate, $CaCO_3$) were commonly added during the process to help precipitation, by increasing the pH to optimal precipitation values, or as extenders to create a pigment with more body and opacity [16]. The addition or not of these materials provided pigments with different colours from light to rosy pink or dark red [22]. In fact, depending on the preparation, brazilwood lakes offered a broad variety of hues. Purchasing the raw material allowed for the artists themselves to prepare the lakes in different ways to obtain the desired colour [15b].

Brazilwood colours were known, since the Middle Ages, to be very fugitive, which has been tested and confirmed recently [13, 15, 28, 29]. Yet, they were much cheaper than the other organic reds and whereas they were unsuitable for works, which were likely to be exposed to light, they may have been appropriate for works such as illuminated manuscripts [15, 23]. Consequently, there is a great diversity of medieval and late-15$^{th}$ and 16$^{th}$ recipes to produce rose-pink brazilwood lakes, sometimes specifically referred to as *rose* or *roseto*, in all sources from European countries [4, 16, 24, 30-32]. These would be prepared from sappanwood (*C. sappan*) or, after 1500, from brazilwood of *C. echinata* or *H. brasiletto*. In more recent times, a brazilwood pigment containing chalk was named *Rose Pink*. According to Harley (2001), this English term appeared in the 18$^{th}$ century and described a fugitive, inferior in colour brazilwood pigment formed by the adsorption of the dye onto the calcium carbonate substrate[7]. In the 19$^{th}$ century, Field[8]

[4] Brazilin and brazilein from brazilwood species are very similar in structure to haematoxylin and haematin, respectively, from logwood (or campeche wood), the American species *Haematoxylum campechianum*, also used as source of dye, which yields colours between blue, violet, grey and black. Brazilwood molecules differ from those of logwood in only one phenolic hydroxyl group. [13, 14]

[5] From the 14$^{th}$ to 17$^{th}$ centuries, kermes, cochineal and madder dyes were probably obtained from dyed textiles [24]. On the other hand, direct extraction from the raw material seems to have been always used for redwoods [24, 25]. However, it does not mean that it could not have been obtained indirectly on occasion as well [16].

[6] Potassium aluminium sulphate dodecahydrated ($AlK(SO_4)_2 \cdot 12H_2O$).

[7] Consequently, it was not a true lake made by means of a chemical reaction, but a less brilliant pigment made by fixing the brazilwood dye on chalk [23]. The light-fastness of pigments prepared in this way is, however, less than for alumina-containing lakes made from the same dyestuff [16, 23].

stated that this pigment was unsuitable for artists [23]. While lac, cochineal and madder were artists' quality colours, brazilwood was included in the economic range and was only preferred to the former when its lower cost was a major factor [23, 35]. In other words, brazilwood was often added as an additive to reduce the cost of manufacture of other types of lake pigment. It was found in 19th century oil paintings, namely those of Vincent Van Gogh (1853 - 1890), together with other organic materials probably to produce a cheaper pigment [36]. As expected, the brazilwood component of these materials has faded and the paintings present significant changes [29].

### 1.3. Documentary Sources

To be able to establish what materials were used to create a given paint and how they were prepared it is necessary to turn to representative historically accurate written sources, such as treatises, recipes' collections and technical handbooks, which can provide essential evidence of materials and techniques contemporary to the artist or work of art [34]. A great number of unique text sources on art technology survive from all periods [37]. In the context of the present work, *Livro de como se fazem as cores* and the Winsor & Newton 19th century archive were chosen because the former is representative of medieval technology and the latter was one of the greatest European artists' materials manufacturer. Together, these sources will allow to understand how the preparation of brazilwood lake pigments has changed.

#### 1.3.1. *Livro de como se fazem as cores*

Medieval artists' recipe texts consist mainly of instructions for the manufacture of materials such as pigments, inks and painting media. The *Livro de como se fazem as cores* (The book on how to make colours) is the only medieval documentary source from Portugal written with Hebrew characters mainly on the art of manuscript illumination and contains important information for medieval technology. The single known copy of this text occupies the first 20 folios of a Hebrew manuscript now at Parma's *Bibliotheca* Palatina (Italy) under the signature Ms 1959. It has been transliterated into the Roman alphabet, translated into English and published for the first time in 1928 by Blondheim. More recently, a new critical edition was made available by Strolovitch (2010). [38, 39]

Plausibly composed by an anonymous Jewish practicing illuminator, this technical text now considered to be from the 15th century is a compilation of 45 chapters/recipes (two of which are missing), comprising both re-worked pre-existing and newly composed material, most of which concern the preparation of materials (inorganic and organic pigments, dyes, binders and varnishes) and the techniques (tempering and mixing) to be used in the illumination of manuscripts [38, 39].

#### 1.3.2. Winsor & Newton Archive of 19th Century Recipes for Artists' Materials

Winsor & Newton was established in 1832 by William Winsor, a colour chemist, and Henry Newton, an artist [34]. Having built their reputation on the quality, reliability, improvement and innovation of their products, this has been the world's leading brand of fine art materials. Their archive from the 19th century includes several hand-written recipe books with records for making pigments, oil and water colours, and other materials, together with details of equipment design, costs and wages, and miscellaneous notes. [40]

[8] George Field (1777 - 1854) was an important British colour manufacturer and supplier of artists' quality pigments in the first half of the 19th century, and collaborator to Winsor & Newton. The best-known 19th century artists' reference manual was Field's *Chromatography or A Treatise on Colours and Pigments, and of Their Powers in Painting & c.*, first published in 1835 [33, 34].

Digital images of individual pages of each of the manuscripts were recently catalogued and indexed into the W&N 19th century database in a project that is part of the Dutch De Mayerne Programme [37, 41]. It is now available internationally at five portals and permission from W&N must be obtained for access due to the commercial sensitivity of some records [42, 43]. This provided an innovative solution for making available information on 19th century artists' materials manufacture [41]. The Researchers' Edition database contains a total of 15003 records distributed in 85 manuscript books, 3579 of which are of restricted access. From that total, 3673 records correspond to pigment manufacture [42].

### 1.4. Case-study: French Books of Hours (15th Century) from *Palácio Nacional de Mafra*

Illuminated manuscripts[9] are one of the most important colour legacies in the European medieval art [45]. Within this context, a book of hour is a medieval manuscript, often illuminated, for private devotion that had particular popularity in France and Flanders [44]. Its illuminations, produced by skilled hands, were richer or poorer according to the patron who commissioned the work [44]. Colours prepared from organic dyes are frequently present in these miniature master pieces as deep, rich glazes or mixed with opaque pigments [46].

Three manuscripts belonging to the 15th century books of hours collection kept in *Palácio Nacional de Mafra* Library (Portugal) were subjected to an interdisciplinary study, with a multi-analytical approach in order to identify the materials and techniques use to produce their colours palette[10]. The three books of hours are French works on parchment (Ms 22, Ms 23 and Ms 24, Table 1). Considering that brazilwood was one of the colour materials most used by 15th century French illuminators, namely by that known as the Boucicaut Master, the manuscripts were integrated in the present work as the perfect case-study for comparison with medieval brazilwood lakes reconstructions [4, 46, 47].

**Table 1.** French books of hours from *Palácio Nacional de Mafra* integrated in the present work.

| Ms 22 | Ms 23 | Ms 24 |
|---|---|---|
| Dated from 1400 to 1420 | Dated from 1410 to 1430 with later additions from 1450 to 1470 | Dated from *ca.* 1420 and 1470 with few later additions |
| 124 folios; 205 x 150 x 43 mm | 129 folios; 200 x 150 x 40 mm | 181 folios; 197 x 135 x 50 mm |
|  |  |  |

[9] The word *illumination* refers to any illustration in manuscripts that highlights or illuminates the written text with luminous colours that way embellishing the manuscript itself [44, 45].

[10] The study of the three manuscripts can be found with detail in Ana Rita Araújo master thesis "Os Livros de Horas (Séc. XV) na Colecção do Palácio Nacional de Mafra: Estudo e Conservação" (2012).

## 2. Experimental Design

### 2.1. Researching the *Livro de como se fazem as cores*

In the 15th century technical text *Livro de como se fazem as cores* there are four brazilwood recipes, chapters 8, 9, 27 and 44 that give instructions to prepare rose colours. The four recipes omit significant details either because the author did not consider them important or because aspects of materials or methodology were so well known and assumed to be obvious to the reader. To try to fill these gaps, besides the English translation of the recipes, their transliteration was also consulted (transcriptions of both may be found in Appendix 1.1). Not only that, information on brazilwood from other reliable documentary sources allowed for a more complete picture to be built [5, 30, 31].

In all four recipes, brazilwood is used as very fine scrapes and three different dye extraction methods are presented (urine, lye and lime). With urine, the pH is assumed to be almost neutral, and with the later two materials, extraction occurs with a basic pH. Alum is always present and with the exception of the last recipe, extenders (mainly chalk) are always added. Chapter 8 is the more complete recipe, referring the precise quantities to use for each ingredient and including a filtration step. The differences observed within the recipes indicate, right way, that different brazilwood lakes can be prepared.

### 2.2. Researching the Winsor & Newton Archive of 19th Century Recipes for Artists' Materials

Research for 19th century brazilwood lake pigments recipes was carried out in the W&N Researchers' Edition database available at DCR-FCT/UNL. A great part of the pigments manufacture records concerns red lakes, e.g. 679 for cochineal, 622 for madder and 56 for lac. Restrictions are mainly applied on those of madder. Only four records, without any restriction, correspond to brazilwood. Found under the research sub-topic "brazil", these four recipes are for preparing Rose Pink pigments. Under the sub-topic "cochineal" one recipe for Wood Purple, based on peachwood was also found. Also, a note from 1849 for a cheap purple lake, found under the sub-topic "purple lake" (prepared from cochineal) refers that "Cheap purple lake might be made by diffusing into the purple liquor some deep coloured rose pink and then throwing down in the usual manner.". Moreover, two other recipes within "brown lake" sub-topic were found to also use peachwood together with other dyestuffs, namely lac and quercitron.

For the purpose of this work three recipes for Rose Pink[11] and the one for Wood Purple were selected (transcriptions may be found in Appendix 1.2). All four are present in book P2, which is dated from 1848 to 1865. In particular, two of them are dated from 1849. In Wood Purple recipe, a comparison with another red dye is made referring that the produced pigment "fades much quicker than cochineal". All four recipes present ingredient quantities and the sequence of steps to follow, with the exception of one (here distinguished by the name "Rose Pink II"). Interestingly, the name used to describe brazilwood species is different in all four recipes. Moreover, in Rose Pink recipe two names are given – brazilletta and India Peach Wood –, each in distinct quantities. All Rose Pink recipes have in common the addition of chalk.

### 2.3. Experimental Design

The experimental design developed in the present work can be divided into three parts as can be seen in Figure 2. All materials, experimental methods and apparatus used are described in Appendix 2.

[11] One Rose Pink recipe was not reproduced since it was only a trial.

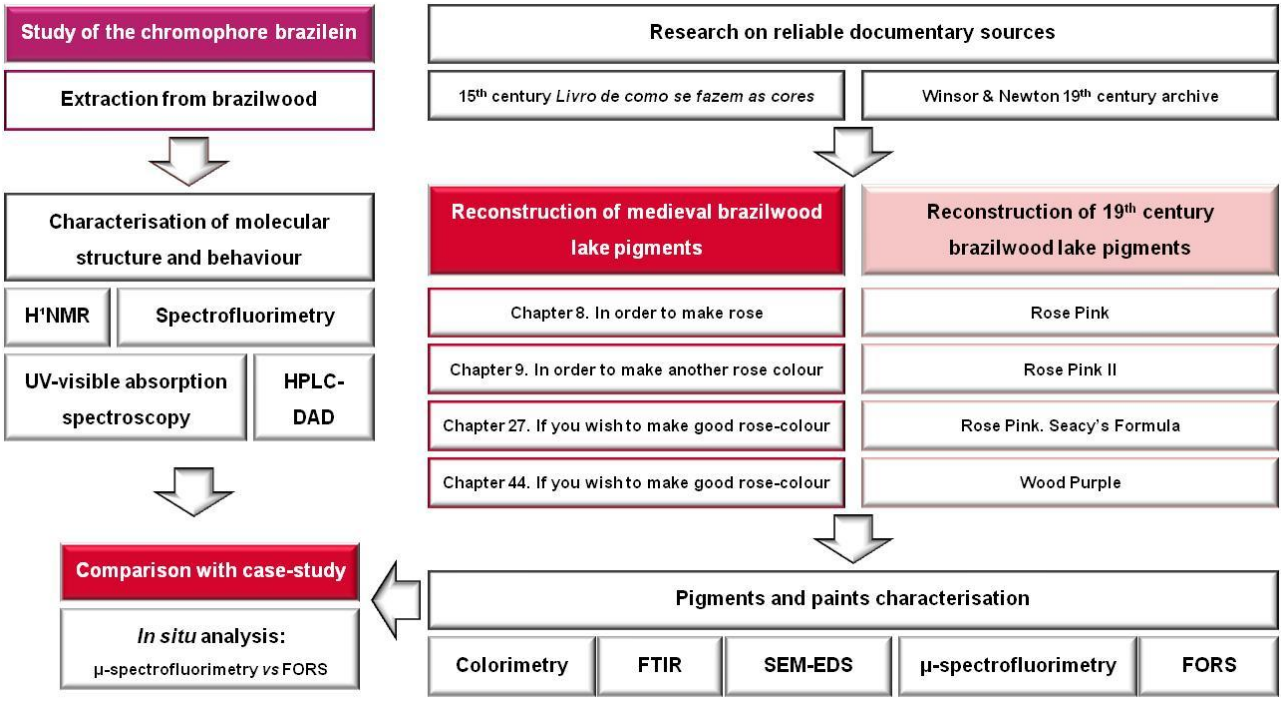


**Figure 2.** Experimental design.

### 2.3.1. The Chromophore Brazilein

Brazilein was isolated from the methanol extract of brazilwood scrapes and its purity was confirmed by $H^1$NMR and HPLC-DAD (Appendix 2.2). The characterisation of brazilein and of its behaviour, including the influence of external factors such as pH and of complexation with $Al^{3+}$, was carried out in both homogenous and heterogeneous media by UV-Vis and fluorescence spectroscopy. Relevant spectra may be found in Appendix 3.

Different species of brazilwood trees were investigated with HPLC-DAD to try to determine if any particular species has a main or any characteristic molecule that can act as a marker as suggested by Nowik (2001). Infrared spectra of the species aqueous extracts were also acquired. Chromatograms and related information may be found in Appendix 4.1 while infrared spectra are present in Appendix 7.

### 2.3.2. Medieval and 19th Century Brazilwood Lake Pigments Reconstructions

Reconstructions were prepared using as much as possible historically accurate methods and materials. Brazilwood from *Caesalpinia echinata*, always in the same starting quantity (5.0 g), was used for all recipes. In particular with respect to the medieval recipes, due to the often unclear instructions and the fact that ingredient's quantities are not obvious each recipe was performed more than once, with ingredient quantities and process variations. Also, medieval lake substrates (without the dye) were produced by precipitating only the hydrated alumina with alum and urine. To confirm the presence of brazilein as the main chromophore in the lake pigments produced, HPLC-DAD analyses was performed on two of the medieval reconstructions (Appendix 4.2). In the 19th century case, "Rose Pink II" and "Rose Pink. Seacy's Formula" were prepared more than once, with different brazilwood species (*C. sappan* and *H. brasiletto*) in order to determine if the use of distinct species would affect the final result[12]. Moreover, in recipe "Rose Pink", where two different names are given to the raw material (each in different quantity), both quantities were considered but only using one brazilwood species. Each reconstruction was ground into paint with a

[12] Because different brazilwood species were available in limited amounts, only these two recipes were prepared more than once.

glass muller and glass slab and painted as tempera on parchment, filter paper and glass slides with both proteinaceous - egg white (glair) and parchment glue - and polysaccharide (gum-arabic) binders.

Conversion of measures from medieval and 19th century sources into S.I. units and scaling down of material quantities from the original to the laboratory scale are found in Appendix 5. Information relating to the preparation of pigments and pH measurements performed during synthesis are described in Appendix 6. All starting and reference materials and binders were analysed by FTIR (Appendix 7).

A total of 22 medieval and 10 nineteenth-century pigments were reproduced. Pigments colour measured using the CIELAB system can be found in Appendix 6 where the values presented are an average for three measurements. FTIR spectra are presented in Appendix 8 (mainly analyses on pigments). SEM-EDS, FORS and μ-spectrofluorimetry analysis were performed in selected reconstructions. Selection was based on paints colours in order to be representative of all the different hues obtained (bluish pinks to dark reds). FORS and μ-spectrofluorimetry spectra can be found in Appendices 9 and 10. FORS spectra were acquired in reflectance but are presented as apparent absorbance, $A' = \mathrm{Log}_{10}(1/R)$. Moreover, although the FORS apparatus used operates between 190–2200 nm, for the purpose of this work only the 350–800 nm range was taken into consideration. Fluorescence emission and excitation data presented were obtained with dichroic filters of 500 and 600 nm, respectively.

The medieval results were compared with that obtained for case-study books of hours. Analysis on manuscripts was performed on light and deep pink and red colours from a total of 12 folios (Table 2; Appendix 11). FTIR, FORS and μ-spectrofluorimetry spectra representative of each folio may be found in Appendices 12, 13 and 14, respectively.

**Table 2.** Multi-analytical approach used to characterise pink and red colours from books of hours*.

| Ms | 22 | | | 23 | | | | 24 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Folio | 22r | 74r | 76v | 2r | 24r | 46r | 129r | 17v | 27r | 53r | 60r | 116v |
| **SEM-EDS** | | | 2 | | | 1 | | | | | | |
| **μ-FTIR** | 1 | | 5 | 2 | 1 | 1 | 1 | | | | 2 | 2 |
| **μ-spectrofluorimetry** | | | 2 | 1 | | 1 | 1 | | | | 1 | |
| ***in situ*** | | | | | | | | 6 | 6 | 6 | 10 | 12 |
| **FORS** | | 3 | 16 | | | | | | | | 4 | 6 |

* Numbers of analyses performed in each folio.

## 3. Results and Discussion

### 3.1. The Chromophore Brazilein

The $H^1$NMR spectrum of the isolated brazilein (Appendix 3, Figure A1) is the same as that presented and described in Berger and Sicker (2009), indicating that the chromophore was successfully extracted from its natural source. HPLC-DAD chromatogram (Figure 3) of brazilein dissolved in methanol, at 450 nm, confirmed that a high purity standard was obtained since only a large retention peak at 14.56 min can be observed, to which the same UV-Vis spectrum with $\lambda_{max}$ at 446 nm is related (Figure 3, inset).

A $pK_{a1}$ = 7 (Figure 4) was obtained by UV-Vis spectroscopy in a water and methanol (70:30, v/v) solution[13]. UV-vis studies are presented in Appendix 3[14].

[13] With respect to UV-Vis pH titrations, further studies are necessary to understand the behaviour of brazilein, namely with respect to the observed increase in absorption of the acidic form when the pH is raised. After this unexpected effect the conversion of AH into $A^-$ was observed with an isosbestic point at 480 nm. (See Appendix 3, Figure A2.)

In acidic solution the maximum absorption occurs at 446 nm (Figure 5A). The acidic species, AH, displays a yellow colour. In a basic environment, the violet anionic form $A^-$ is present and a red shift is observed ($\lambda_{max}$ = 540 nm). When applied in filter paper, the protonated and deprotonated forms of brazilein also present different excitation and emission spectra (Figure 5B). For the protonated form of brazilein maxima of 475 and 536 nm are obtained for excitation and emission spectra, respectively. For the $A^-$ form, maxima of 548 and 560 nm are obtained, respectively. The latter was the only species for which a good match between excitation and absorption spectra was observed. In acidic media, excited state reactions are effectively competing for the fluorescence emission of AH.

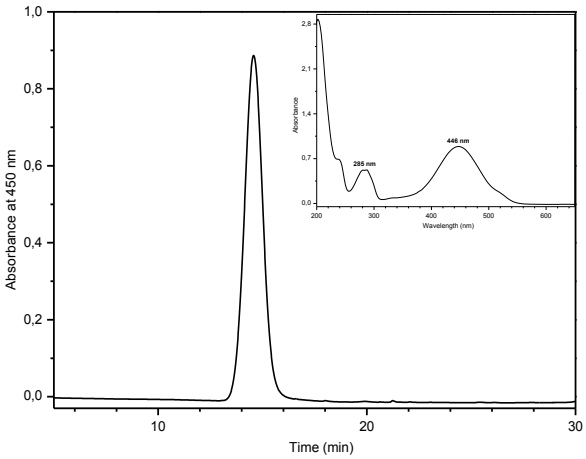


**Figure 3.** HPLC-DAD chromatogram of brazilein at 450 nm (inset: UV-Vis spectrum at 14.56 min).

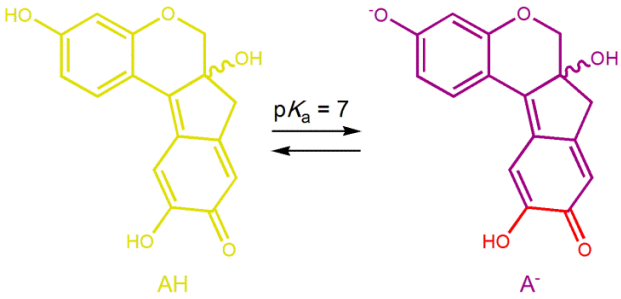


**Figure 4.** p$K_{a1}$ scheme for the weak organic acid brazilein where AH and $A^-$ represent the acidic and basic species, respectively; the α-hydroxyquinone moiety is highlighted in red.

When the dye is complexed with $Al^{3+}$, changes in absorption as well as excitation and emission properties are observed. Complexation with the metal cation results in an absorption shift to shorter wavelengths and the solution turns to red, with a maximum at 510 nm (Figure 6A). This is different from what obtained for $A^-$ ($\lambda_{max}$ = 540 nm), suggesting that coordination takes place in the α-hydroxyquinone moiety[15] [49]. Excitation and emission spectra of the $Al^{3+}$ complex in the solid state present maxima at *ca.* 520 and 585 nm, respectively (Figure 6B). Absorption maximum of the solid state corresponds to that of excitation (Figure 6B).

A full rationale of the results obtained, mainly with respect to those of fluorescence, may be only possible after a full understanding of the photophysics of both brazilin and brazilein. However, this discussion is outside the scope of this work.

[14] Brazilein molecule was found to be very stable in methanol, without any variation in maxima wavelength or spectra shape, and intensity remaining constant. In a mixture of water and methanol (70:30, v/v) at pH 6.1 no change in maxima wavelength or spectra shape is observed either. However, in this case, a large decrease in intensity is noticed in the first hours. The same thing happens in a mixture of water and methanol (70:30, v/v) at pH 2.9, although the decrease in intensity is not so significant.

[15] Wongsooksin *et al.* (2008) have proposed that brazilein coordinates as two bidentate ligands through the ionized hydroxyl group and carbonyl oxygen to $Al^{3+}$ with two water molecules acting as co-ligands to complete an octahedral configuration [48]. Still, a better molecular understanding of mordant-dye interactions is needed.

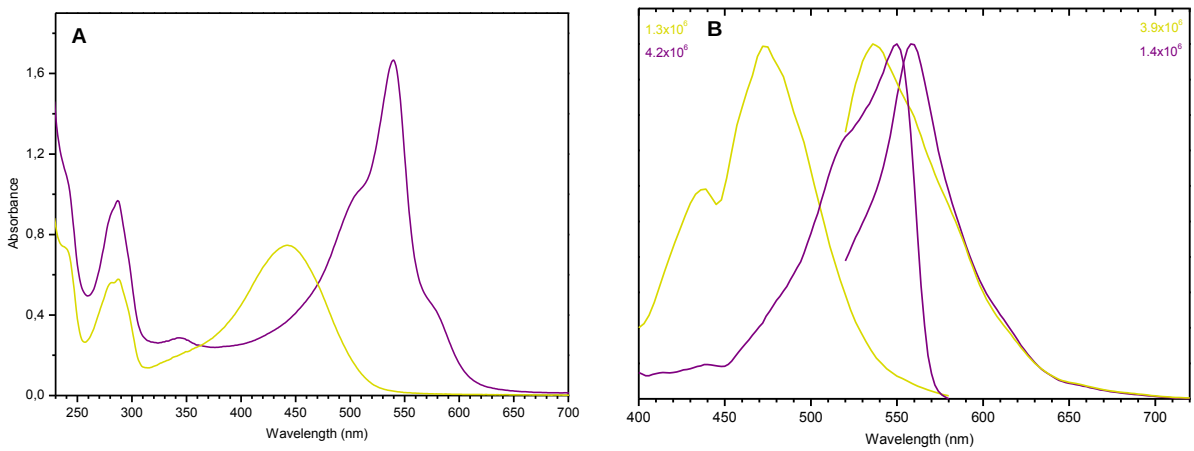


**Figure 5. A)** UV-Vis spectra of brazilein in a mixture of water and methanol (70:30, v/v) at pH 2 (— AH) and 8 (— $A^-$); **B)** Emission and excitation spectra of brazilein applied on filter paper for AH (—) and $A^-$ (—).

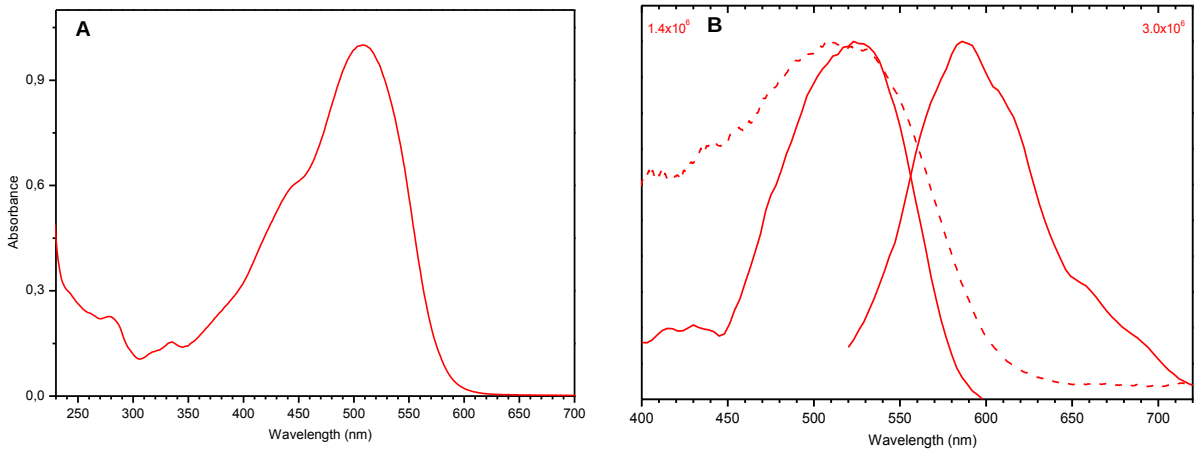


**Figure 6. A)** UV-Vis absorption spectra of brazilein-$Al^{3+}$ complex in a mixture of water and methanol (70:30, v/v) at pH 4.2; **B)** Emission and excitation (—) and absorption (– –) spectra of brazilein-$Al^{3+}$ complex applied on filter paper.

### 3.2. Medieval and 19th Century Brazilwood Lake Pigments Reconstructions

Based on the treatises and references consulted [16, 30, 31], the preparation of a lake pigment can be generally described by the scheme presented in Figure 7, where the filtration step is however optional and the addition of extenders is variable. If no dye is present, the process results in the formation of a fairly white amorphous precipitate based on aluminium hydroxide, $Al(OH)_3$ [25] (Appendix 7, Figure A15).

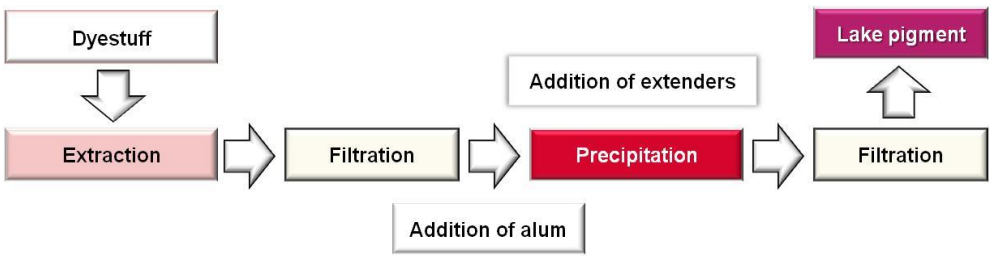


**Figure 7.** The lake pigment process.

The *Livro de como se fazem as cores* and the W&N 19th Century Archive, two technical documentary sources distant from each other in time, present recipes for the preparation of brazilwood lake pigments. A description of the historical recipes reproduced and the results obtained will be discussed. In particular, the

reproductions will allow to study the effect of the different procedures, the different ingredients used that can act as precipitating agents, chelating ions, extenders or buffers, and of the pH.

### 3.2.1. Reconstructions based on *Livro de como se fazem as cores*

The substrate without the dye was prepared and characterised by FTIR. Amorphous precipitate was obtained when alum was added to a urine solution with pH 7.0 and sodium hydroxide was used to obtain a neutral final pH (Figure 8). Infrared analyses of the precipitate revealed the presence of a sulphate ($SO_4^{2-}$) component due to the bands at 1120 and 988 $cm^{-1}$ characteristic of S-O vibrations (Figure 9A) [25, 27]. Broad bands occurring at 3400 ($\nu$(OH)) and 1647 (OH deformation) $cm^{-1}$ are associated to the amorphous nature of the aluminium hydroxide precipitated solid phase [25, 27]. A band that can be attributed to Al-O vibrations may eventually be present in the 750-400 $cm^{-1}$ region [27]. Infrared spectrum of the precipitated brazilein-$Al^{3+}$ complex (Figure 9A) reveals the same absorption bands.

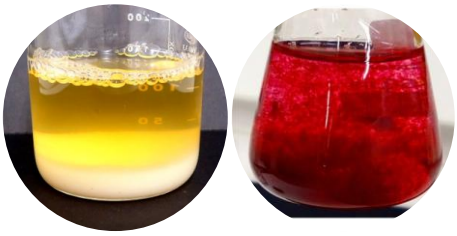

**Figure 8.** Lake formation without and with dye: urine and alum at pH 7.0 (left) and brazilein in methanol with alum at pH 6.0 (right).

Moreover, to help in the interpretation of the results obtained with medieval reconstructions, infrared spectrum of brazilein and the wood of *Caesalpinia echinata* were also acquired (Figure 9B and Appendix 7, Figure A21). Brazilein spectrum presents several sharp bands of variable intensities between 1600 and 668 $cm^{-1}$, which can be attributed to vibrations such as C=C stretching, in-plane CH bending and deformation and out-of-plane CH deformation, characteristic of heterocyclic compounds with aromatic rings [50].

HPLC-DAD analyses performed on two of the medieval reconstructions, which are presented in Appendix 4.2, confirmed the presence of brazilein as the main chromophore present in the pigments.

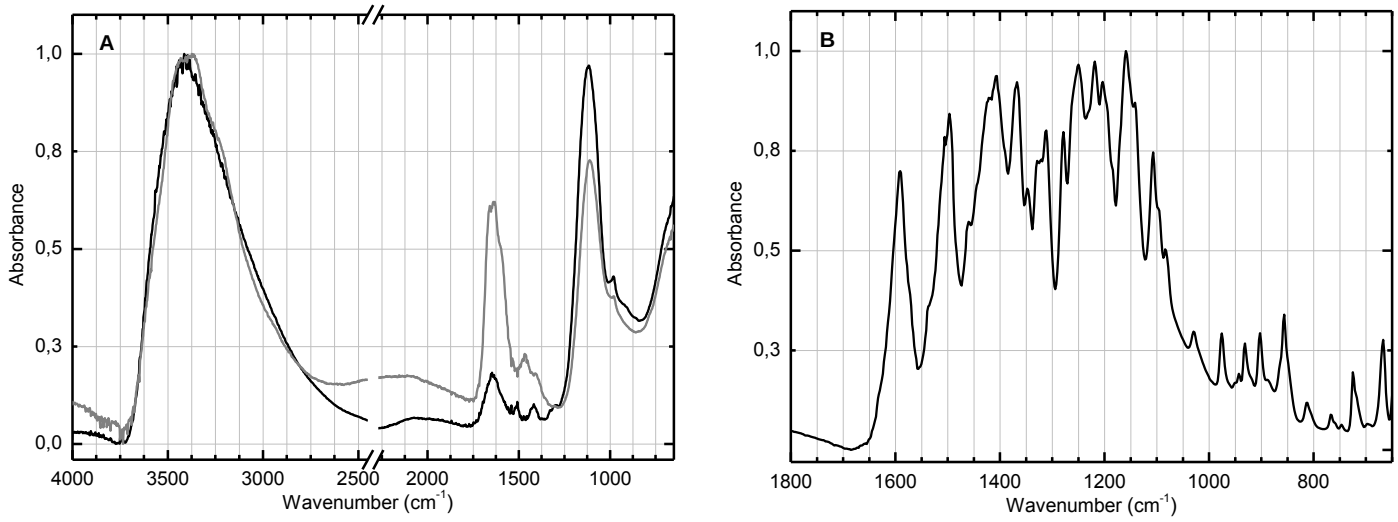


**Figure 9.** Infrared spectra of **A)** brazilein-$Al^{3+}$ complex precipitated at pH 6.0 (**—**) and lake substrate precipitated at pH 7.0 (—) and **B)** brazilein from 1800 to 650 $cm^{-1}$ (spectrum from 4000–650 $cm^{-1}$ may be found in Appendix 7, Figure A21.B).

Besides chapter 8, all three medieval brazilwood recipes from *Livro de como se fazem as cores* have in common the inexistence of precise quantities for each ingredient used. Chapters 9, 27 and 44 refer only subjective quantities such as “as much as you need”, “take a piece of” and “put in a little”. It is also not

clear if brazilwood was used in the form of scrapes or powder[16]. Recipes suggest to “scrape it very fine” or “scrape it well”. With the exception of recipe 8, no recipe refers the filtration step. Straining the dyestuff solution could be such an obvious thing to do that it was thought to be unnecessary to write down and was therefore omitted on purpose from the procedure. There is also the possibility that the solution was in fact not filtrated and in that case brazilwood scrapes or powder would be present in the final pigment as will be further discussed.

The main difference between recipes concerns the pH of the extraction solution, which determines the extraction times (ranging from a couple of hours, or less, to a few days). In chapters 8 and 27 dyestuff extraction is made with urine[17] and the solution, to which alum is added, is allowed to stand for three days. pH measurements in the laboratory of the fresh urine used indicate this is not an alkaline solution but presents pH values that are within the normal physiological range of 5.6 and 6.8 [51, 52]. Experiments showed that higher pH values can however be obtained with stale urine[18]. In chapters 9 and 44, extraction is carried out in lye (left to boil) and lime, respectively, which give very alkaline solutions.

With respect to the ingredients, alum is present in the four recipes and chalk is added in chapters 9 and 27. Chapter 8, in particular, refers the addition of lead white and indicates that the dye solution is filtrated over a chalk or gypsum stone.

Reconstructions were prepared as described in Figure 10 with slight variations. Ground brazilwood was always used. Despite chapter 44 indicates that brazilwood is macerated in lime, also in this case the raw material was ground and the dye extracted in solution. Urine with neutral pH and lye and lime solutions with pH’s values between 11 and 12 were used. In some cases, no filtration step after extraction was performed yielding a final pigment with the ground wood in it, which turned out to be difficult to paint with. In chapter 8, chalk was used to make a bowl but, in most experiences a gypsum bowl, which revealed to be the most efficient, was used (Figure 11). Centrifugation was performed to optimise the pigment yield. Table 3 presents information concerning the most representative reconstructions (each reconstruction is named for “chapter x, experience x”), and a more complete description of all pigments produced may be found in Appendix 6.1.

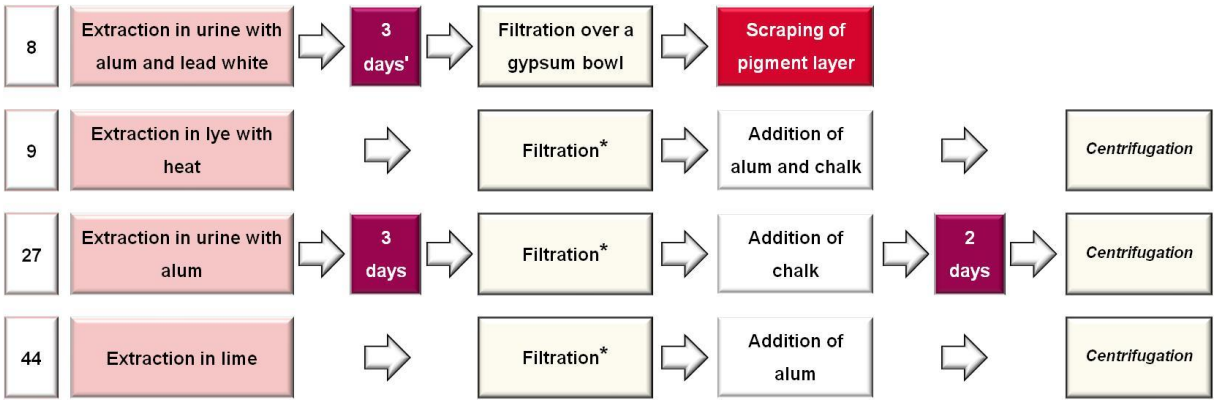


**Figure 10.** Sequence of steps followed for the preparation of medieval brazilwood pigments according to chapters 8, 9, 27 and 44 from *Livro de como se fazem as cores*; (´) stirring 5/6 times each day; (*) filtration was not mentioned in the original recipes.

[16] However, as stated before, it is known that some medieval dyers ground the wood into powder in mortars with pestles [13].

[17] Human urine comprises high concentrations of nitrogen (N), in the form of urea, phosphorous (P), potassium (K), sodium (Na) and chloride (Cl). Its composition varies from person to person and accordingly to several external factors. [51]

[18] During storage, urine is subject to spontaneous processes such as urea hydrolysis that change its composition significantly. Urea is rapidly degraded, which may elevate pH values up to 9. [52]

In chapter 8, whenever urine is added to brazilwood with lead white and alum, effervescence occurs, indicating $CO_2$ release (eq. 4) and the solution turns to a red orange/brown colour, getting redder with time. In chapters 9 and 44, when lye and lime are poured into the brazilwood, the solution stays immediately red and starts to turn to dark purple due to the basic environment provided by the alkaline solutions. Also in chapter 9, effervescence is observed when chalk is added to the acidic dye solution. $CO_2$ release and the buffering effect may be explained by the acid-base equation established by $CO_3^{2-}$ [53]:

$$CaCO_3\,(s) \rightleftharpoons Ca^{2+}\,(aq) + CO_3^{2-}\,(aq) \qquad \text{(eq.1)}$$

$$CO_3^{2-}\,(aq) + H_3O^{+} \rightleftharpoons HCO_3^{-}\,(aq) + H_2O \qquad \text{(eq.2; p}K_a\text{ 10.3)}$$

$$HCO_3^{-}\,(aq) + H_3O^{+} \rightleftharpoons H_2CO_3\,(aq) + H_2O \qquad \text{(eq.3; p}K_a\text{ 6.38)}$$

$$H_2CO_3\,(aq) \rightleftharpoons CO_2\,(g) + H_2O \qquad \text{(eq.4)}$$

The pH of extraction solutions in chapter 8 and 27 is around 4.5. In this case, urine is not acting as a buffer as was expected[19]. With lye solutions of pH 11-12, the pH of extraction can vary between 8 and 10. In chapter 44, lime solution with pH 12 gives a pH of extraction of 10. With respect to final pH values, in chapters 9, 27 and 44, depending on the quantity of alum and chalk added, it varies between 5 and 7. Considering that $Al^{3+}$ is a Lewis acid, it was observed that as expected the more alum is added, the lower the pH is.

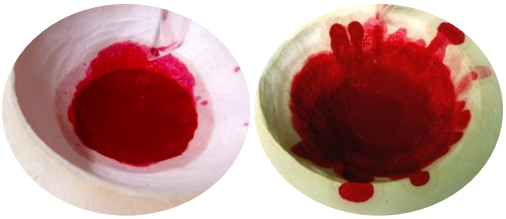

**Figure 11.** Filtration over a bowl made of gypsum (chapter 8, experience 5).

In chapter 8, when the dye solutions are filtered to a gypsum bowl it is perfectly absorbed forming a reddish solid layer on the top of the gypsum, which is scraped. On the other hand, when it is filtered above a bowl made of chalk, it is only partially absorbed. Practical experiences revealed also that if the solution is directly poured into the top of the gypsum bowl (without previous filtration) to be absorbed, it is then more easy to remove the wood scrapings and scrape only the pigment precipitated on the bowl surface.

**Table 3.** Information on the preparation of selected reconstructions from *Livro de como se fazem as cores* and their colour coordinates.

| Chap. | Exp. | Urine | Lye | Lime | Alum | Chalk | Gypsum | Lead white | pH of extraction | Final pH | Filtration | L*a*b* † (Ga) | L*a*b* † (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 1 | • | | | • | | • | • | 4.3 | | | 38 32 15 | 53 25 9 |
| | 5 | • | | | • | | • | • | 5.3 | | • | 53 33 3 | 50 30 -8 |
| | 6 | • | | | • | | • | • | 4.2 | | • | 61 29 9 | 55 31 -6 |
| 9 | 2 | | • | | • | • | | | 9.0 | 6.2 | • | 37 25 2 | 31 20 -2 |
| | 4 | | • | | • | • | | | 10 | 7.0 | | 33 22 -3 | 31 22 -5 |
| | 5 | | • | | • | • | • | | 10 | | • | 50 24 1 | 41 24 -5 |
| 27 | 1 | • | | | • | • | | | 4.0 | 6.1 | | 62 17 -13 | 63 18 -12 |
| | 3 | • | | | • | • | | | 4.3 | 5.8 | • | 63 28 -7 | 62 27 -11 |
| 44 | 1 | | | • | • | | | | 9.7 | 7.0 | | | |
| | 4 | | | • | • | | | | 10 | 6.1 | • | 39 42 17 | 36 44 15 |

† "L*a*b* (Ga)" refers to colour coordinates of pigments painted with gum-arabic and "L*a*b* (Ew)" refers to those painted with egg white. In both cases, paints were applied on parchment.

[19] Considering previous experiments carried out in the Technical Art History course by Conservation and Restoration master students.

Variables such as the ingredients added and their quantities and the pH of extraction and precipitation proved to have influence in the colour of the pigment obtained, varying from pink to red and purple. In particular, the addition of higher quantities of chalk produces colours with higher blue component (lower b* value). Depending on this factor, colours obtained can be either pale to bright pink (chapter 27, with lower a* and b* values) or light to deep purple (chapter 9). On the other hand, chapter 8 colours are more diverse varying from pink (b* negative) to red (higher yellow component), while those from chapter 44 have higher b* values tending towards red-purple colours. Concerning the luminosity, chapters 8 and 27 produce colours with higher L* values than chapters 9 and 44, which produce darker hues due to the lower quantities of extender added. These latter two recipes resulted in lower pigment yields as well. In general, when painted with egg white, colours are generally bluer (b* negative) than when the binder is gum-arabic. This effect has been also observed in the study carried out by Roger, Villela-Petit & Vandroy (2003).

Infrared analysis of pigments and paints revealed mostly the presence of the extenders and binders. However, identification of the dye, which is usually not possible due to its low concentration, could also be made. Nonetheless, possible contributions from more than one material made it difficult to be sure what the source of some bands might be. With respect to reproductions based on chapter 8, infrared spectra mainly composed of calcium carbonate (chalk) or calcium sulphate dihydrate (gypsum), depending on the ingredient added during manufacture, were detected (Appendix 8.1, Figures A22.B and A24.A). Lead white, added in all chapter 8 experiences in minor quantities, is never detected in infrared analysis, possibly due to its consumption evidenced by the $CO_2$ release. In Appendix 8.1, Figures A22.A and A23, gypsum presents its characteristic absorption bands at 3404 $cm^{-1}$ ($\nu$(HO)), 1134 $cm^{-1}$ ($\nu_{as}(SO_4^{2-})$) and 670 $cm^{-1}$ ($\delta_{as}(SO_4^{2-})$) [8]. In this case the extra signs on the OH stretching area and the broadening of the band at 1134 $cm^{-1}$ can be due to the aluminium hydroxide substrate. Spectra of chapter 9 pigments reveal both the presence of the chalk added, with absorptions bands at 1408 $cm^{-1}$ ($\nu_{as}(CO_3^{2-})$), 875 and 713 $cm^{-1}$ ($\delta_{as}(CO_3^{2-})$), and the dyestuff extract (Appendix 7, Figure A19.B) with broad band centered at 3400 $cm^{-1}$ and well defined bands between 1610 and 820 $cm^{-1}$, as can be seen in Appendix 8.1, Figures A24.B. When the filtration step is omitted during the synthesis of the pigment, in the respective infrared spectrum the cellulose pattern (bands at 1165, 1115 and 1055 $cm^{-1}$) and the broad band at 3400 $cm^{-1}$ can be detected together with the extender (chalk) indicating the presence of the wood itself (Figure 12A) [54]. Chapter 27 reproductions spectra show mainly the presence of chalk (Appendix 8.1, Figure A27.A). Interestingly though, one of the spectra reveals the presence of gypsum as well as that of chalk indicating that the former was produced during pigment synthesis (Figure 12B). This may be explained by the disappearance of $CO_3^{2-}$ in the form of $CO_2$ and the simultaneous presence of $SO_4^{2-}$ ions from alum and $Ca^{2+}$ from calcium carbonate in solution, at acidic conditions (when chalk is added the dye solution containing alum is at pH 4). In spectra from chapter 44 reproductions both the pigment's amorphous substrate, with the S-O vibrations characteristic bands at 1120 and 988 $cm^{-1}$, and dye (1500, 1415 and 1300 1250 $cm^{-1}$) can be detected (Appendix 8.1, Figure A28). The identification of these bands was possible since no extenders were added, therefore there is no overlapping of their characteristic bands.

When infrared analysis is performed on paints (Figure 12), the pigment spectra can be totally or partially masked by that of the binder, either gum-arabic or egg white, depending on how much is added. The polysaccharide binder can be identified by a broad band centred at 3380 $cm^{-1}$ resulting from the OH stretching of the hydroxyl groups and bound water, and also by the vibrations at 1610 $cm^{-1}$ ($\nu_{as}$(C=O)) and 1415 $cm^{-1}$ ($\nu_s$(C=O)) from the carboxylate group. Most important when characterising such binder are the

glycoside bonds that present skeletal C-O and C-C vibration bands in the region 1200-1000 $cm^{-1}$. The following vibrations can be assigned: ν(COC) at 1150 $cm^{-1}$, ν(CC(CO)) at 1120 and 1040 $cm^{-1}$, and δ(OH) and ν(CO) at 1080 $cm^{-1}$. On the other hand, the proteinaceous binder is detected by the presence of the polyamide pattern: amide I (ν(C=O) at 1650 $cm^{-1}$), amide II (ν(CN) and δ(NH) at 1540 $cm^{-1}$) and δ(CN) at 1450 $cm^{-1}$, and also by the ν(OH) and ν(NH) at 3400-3000 $cm^{-1}$. [8, 45]

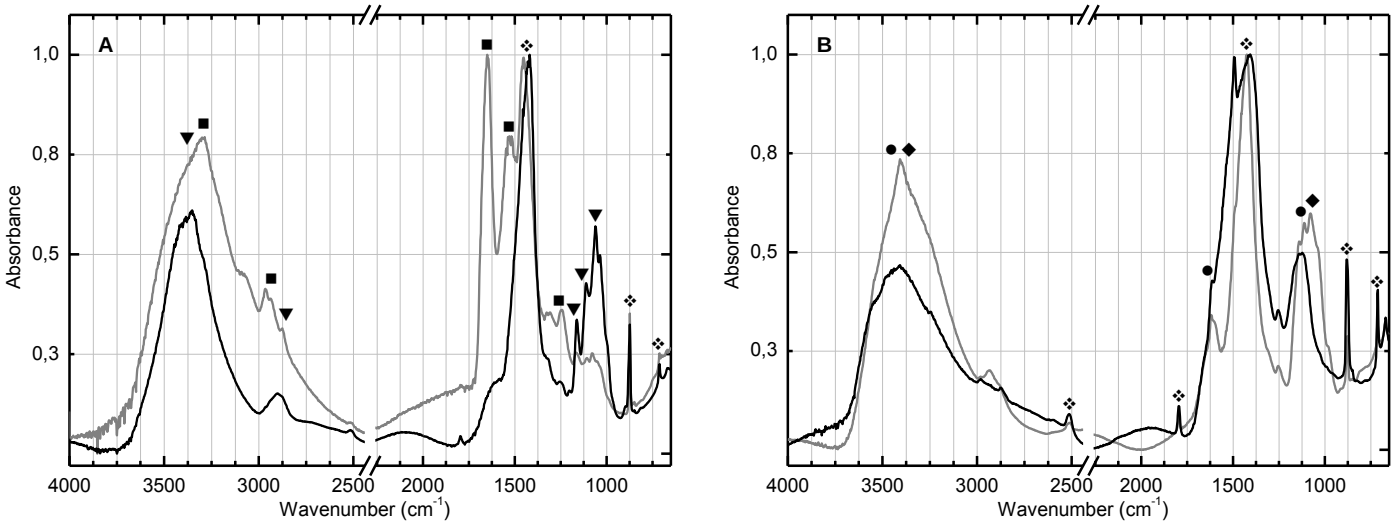


**Figure 12.** Infrared spectra of **A)** chapter 9, exp. 4 pigment (**—**) and paint with egg white (—) and **B)** chapter 27, exp. 3 pigment (**—**) and paint with gum-arabic (—); in which the main absorptions are assigned to: (❖) $CaCO_3$, (●) $CaSO_4 \cdot 2H_2O$, (▼) cellulose, (◆) polysaccharide binder and (■) protein binder.

SEM-EDS analysis was performed in one of the experiences carried out following chapter 9 indications but filtrating the dye, alum and chalk solution over a gypsum bowl as in chapter 8. The presence of calcium carbonate in the form of particles with rough appearance, irregular shape and large dimensions was confirmed (Figure 13). Similarly, the presence of gypsum was detected, confirmed the information obtained with FTIR analyses. This compound was found to be present in the form of particles that seem to be "built up in layers", of large dimensions, irregular in shape and not-elongated. Usually powder gypsum particles present a rough and elongated shape of large dimensions [3]. This difference may eventually be justified by the fact that the pigment was strained over a bowl made of gypsum to dry, and then scraped with a spatula. Besides the elements sulphur (S), calcium (Ca), carbon (C) and oxygen (O), EDS detected the presence of aluminium (Al), sodium (Na) and silicon (Si) in minor quantities. While the former are mainly related to the extenders added during pigment manufacture and to the substrate formed, and Al is associated with the mordant, the latter are most probably related to the natural organic dye [25].

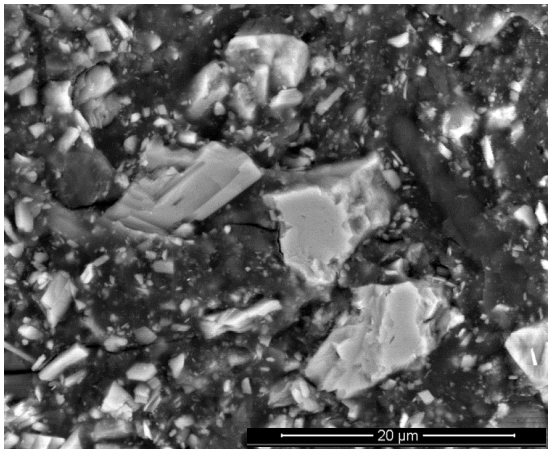


**Figure 13.** SEM image in BSE mode of chapter 9, exp. 5 paint µ-sample.

µ-spectrofluorimetry and FORS were used to understand the potential of each technique in the analysis and characterisation of the dye present in the paints. FORS spectra were acquired in selected pigments

from chapter 8 and 9 painted with gum-arabic, egg white and parchment glue on parchment. Fluorescence analyses were performed on pigments painted with the same binders on filter paper and parchment.

FORS spectra are identical despite the different recipes and ingredients used to produce the pigments and the various colours obtained with the different paints. A shoulder at 355 nm and an absorbance band presenting a small shoulder at *ca.* 520 nm and with maximum at 560 nm are present in all spectra (Figure 14A, Table 4). This is not consistent to the binder effect registered by Roger, Villela-Petit & Vandroy (2003) that observed different absorption maxima for paints with the polysaccharide binder (525 nm) and with egg white (550-60 nm). On the other hand, similarly to what is reported by Bisulca *et al*. (2008), best results were obtained at low paint concentrations. Also, FORS spectra of lakes based on carminic acid and alizarin, acquired during the latter study, reveal distinct features of those herein obtained.

With respect to fluorescence analysis, in general, good excitation and emission spectra were obtained, which shape is also similar despite the different recipes and paints' binder and subtract (Figure 14B; Table 4). Excitation spectra present a more or less large band with maximum wavelength at *ca.* 553 nm (varying between 552 and 560 nm). Emission spectra present maxima at *ca.* 585-90 nm, although in a few cases (not reported) these values varied between 580 and 610 nm.

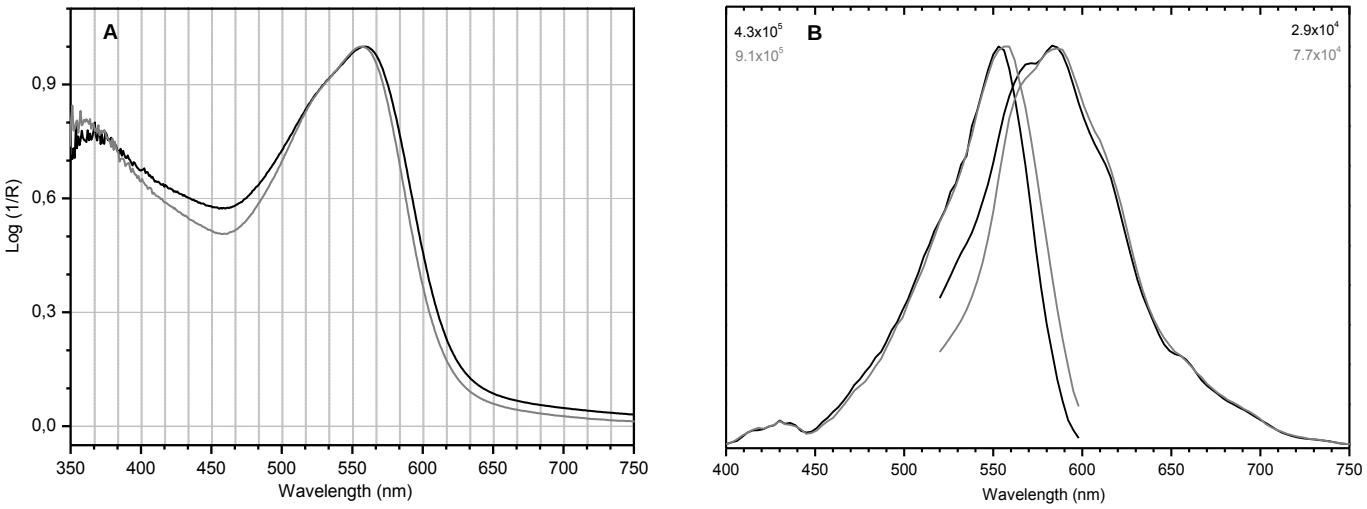


**Figure 14. A)** Absorbance spectra of chapter 8, exp. 6 (—) and chapter 9, exp. 5 (**—**) paints on parchment; **B)** Emission and excitation spectra of chapter 8, exp. 6 paint with gum-arabic on filter paper (—) and chapter 9, exp. 5 paint with gum-arabic on filter paper (**—**).

In both techniques it was not possible to discriminate between the different extenders in the paint for the spectra obtained are identical whether gypsum or chalk has been added during pigment synthesis. Also, no binder effect was observed. On the other, as could be expected, the excitation spectra match very closely those of absorbance and, for each technique there is a shift of *ca.* 30 nm from the main chromophore - the brazilein-$Al^{3+}$ complex - in filter paper to the reconstructions. While Figure 6B shows the absorbance and excitation of the complex at 520 nm, absorbance and excitation of reconstructions is at 553-60 nm (despite this difference, fluorescence emission maximum is kept at 585-90 nm). This indicates that neither technique is giving exclusive information on the dye-metal complex. Instead, they detect the signal produced by the pigment matrix, which proved to be characteristic of the brazilwood lake pigment.

All the information obtained provides standards similar to what may have been used in the production of Portuguese medieval illuminations. These can be used as references in appropriate comparison with any case study and can give some insight into the importance of *Livro de como se fazem as cores* in the context of European medieval technology.

**Table 4.** Absorption (FORS), excitation and emission maxima for brazilwood lake reconstructions painted with gum-arabic and egg white.

| Chapter | 8 | | | 9 | | 27 | | | 44 | |
|---|---|---|---|---|---|---|---|---|---|---|
| experience | 3 | 5 | 6 | 2 | 5 | 1 | 3 | 4 | 1 | 4 |
| **Absorption λmax (nm)** | - | - | 560 | - | 560 | - | - | 560 | - | - |
| **Excitation λmax (nm)** | 553 | 553 | 557 | 555 | 553 | 553 | 553 | 556 | 553 | 553 |
| **Emission λmax (nm)** | 585 | 586 | 585 | 580 | 585 | 585 | 580 | 585 | 580 | 583 |

### 3.2.2. Reconstructions based on Winsor & Newton 19th Century Archive

The four 19th century recipes, all similar in procedure, were fairly straightforward to interpret. The main concerns were related with recipe Rose Pink II that presents no sequence of steps. Moreover different names were used to describe the brazilwood species. There is also no indication on how the natural dyestuff would be used, if in the form of scrapes or very well ground. However it is said that in this period brazilwood was ground in mills [22].

All four recipes include alum and one carbonate compound to act as buffer and to provide opacity. Dye extraction is always carried out with boiling water, without any pH control, and the filtration step is always present. pH control is obtained at the final step by the simultaneous addition of $Al^{3+}$, a Lewis acid, and the carbonate ion, which allows to establish the acid-base equilibriums of equations 2 and 3.

Reconstructions were prepared as described in Figure 15. With the exception of Rose Pink, in which a minor quantity of brazilwood was used, the raw material was always used with the same starting quantity and also ground in a coffee grinder. Water used had a pH of 6. Rose Pink II recipe was performed twice, using both *Caesalpinia echinata* and *Caesalpinia sappan* brazilwood species (the latter is identified as Rose Pink II (b)). Rose Pink Seacy's recipe was also performed twice, using *Caesalpinia echinata* and *Haematoxylum brasiletto* (the latter is identified as Rose Pink Seacy's (b)). Centrifugation was performed to optimise the pigment yield. Table 5 presents information on the reconstructions that were carried out and a more complete description may be found in Appendix 6.2.

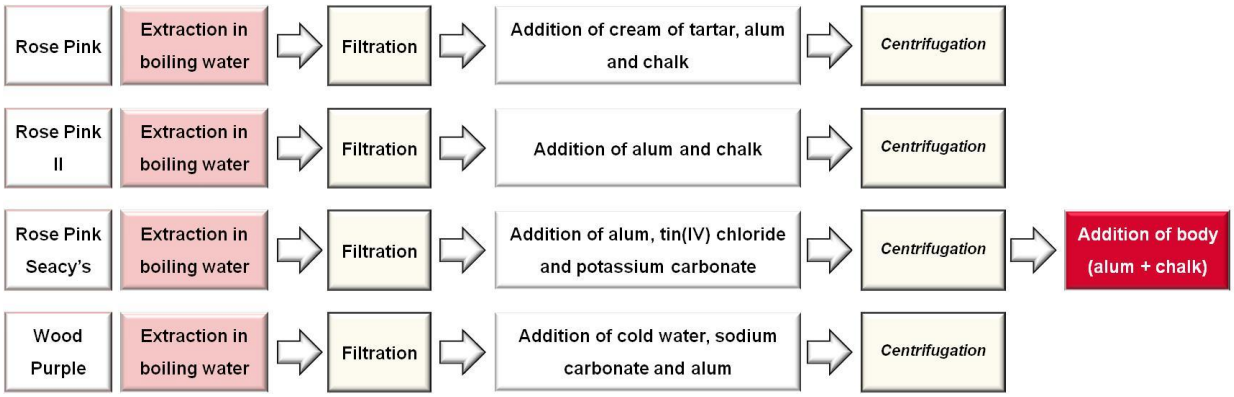


**Figure 15.** Sequence of steps followed for the preparation of 19th century brazilwood pigments according to recipes from the 19th century W&N archive.

The extraction in water yields a solution with pH between 4 and 5. In Rose Pink recipe the addition of cream of tartar (potassium hydrogen tartrate, $C_4H_4KO_6$) turns the solution into yellow (pH 3.5), which, when alum is added changes to red (indicating the formation of the dye-$Al^{3+}$ complex), at the same time that a

decrease in the pH is observed (pH 2.5). According to Golikov (2002), cream of tartar may act as an additional ligand on the formation of the dye-$Al^{3+}$ complex. The further addition of a great quantity of chalk leaves the solution with a light pink colour (pH 6.0). In Rose Pink II, which is the simplest recipe only alum and chalk are added. In both recipes, the addition of chalk contributes to pigment precipitation, increasing the pH. The pigments obtained present a light and strong pink colour, respectively.

**Table 5.** Information on the preparation of selected W&N reconstructions and their colour coordinates.

| Recipe | Boiling water | Cold water | Alum | Chalk | Cream of tartar | Permuriate of tin(IV) | Potassium carbonate | Carbonate of soda | pH of extraction | Final pH | Filtra-tion | L*a*b* † (Ga) | L*a*b* † (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rose Pink | • | | • | • | • | | | | 4.2 | 5.9 | • | 81 23 3 | 78 28 3 |
| Rose Pink II | • | | • | • | | | | | 4.5 | 6.8 | • | 39 46 12 | 46 46 2 |
| Rose Pink II (b) | • | | • | • | | | | | 5.0 | 6.7 | • | 42 45 10 | 39 40 7 |
| Rose Pink Seacy's | • | | • | • | | • | • | | 4.5 | | • | 68 39 10 | 76 27 12 |
| Rose Pink Seacy's (b) | • | | • | • | | • | • | | 4.4 | | • | 61 44 10 | 65 39 8 |
| Wood Purple | • | • | • | | | | | • | 4.7 | 7.0 | • | 38 47 9 | 45 45 1 |

† "L*a*b* (Ga)" refers to colour coordinates of pigments painted with gum-arabic and "L*a*b* (Ew)" refers to those painted with egg white. In both cases, paints were applied on parchment.

In Rose Pink. Seacy's Formula recipe, besides alum, permuriate of tin (interpreted as tin(IV) chloride pentahydrated, $SnCl_4 \cdot 5H_2O$) is also added as a precipitating agent. This produces an aluminium- and tin-containing brazilwood lake pigment [36]. While the addition of alum to the dye solution turns it into a strong red colour, the addition of tin(IV) chloride turns the solution into a brighter red (Figure 16) and precipitation of a red pigment occurs at very acidic conditions (pH 0.5). This result suggests that tin is able to form complexes and precipitate at acidic pH's, unlike aluminium that requires a neutral environment to precipitate. However, a pinkish final colour is obtained by the addition of a "body" prepared by the addition of an alum solution to a calcium carbonate paste.

Wood Purple is the only recipe in which chalk is not added. In this case, following the original ingredients quantities, a final pH of 9.7 is obtained and despite the final comments present in the recipe, the result is colourless. This probably occurs due to the buffering effect of the addition of sodium carbonate previously to the addition of alum. Adding a higher quantity of alum, which lowers the final pH to 6.9, a purplish pigment is obtained.

In terms of L*a*b* colour coordinates, with the exception of Rose Pink pigments, all fall inside the ranges: a* between 39 and 47, and b* between 9 and 12. Rose Pink alone yields colours with a* = 23 and b* = 3, probably as a consequence of the high amount of chalk added. The latter and Rose Pink Seacy's Formula present the higher L* values. When a different brazilwood species is used (*Caesalpinia sappan* and *Haematoxylum brasiletto* in Rose Pink II (b) and Rose Pink Seacy's (b)), there is no significant difference in the result[20].

The pigments obtained display similar infrared spectra, which show only the presence of extenders (Figure 17A), with a high content of calcium carbonate, with the characteristic absorptions bands at 1408, 875 and 713 $cm^{-1}$, and most interestingly of gypsum (bands at 3404, 1134 and 670 $cm^{-1}$), which was not

[20] The chromophore, brazilein has been found to be chemically the same despite the plant source/species (see Appendix 4.1).

added during pigment synthesis [8]. As previously explained, the formation of this compound may be explained by the simultaneous presence of $SO_4^{2-}$ ions from alum and $Ca^{2+}$ from calcium carbonate in solution, at acidic conditions. On the other hand, no other ingredient originally added is visible in the infrared spectra.


**Figure 16.** Addition of the different ingredients to the dye solution in Rose Pink Seacy's recipe with the respective colour change.

Wood Purple pigment infrared spectrum (Figure 17B) is completely different from the previous ones, not showing the presence of any starting material but apparently revealing the presence of dawsonite, a crystalline sodium aluminium hydroxyl carbonate, $NaAl(CO_3)(OH)_2$, formed from the reaction between amorphous aluminium hydroxide and sodium carbonate at neutral pH (Appendix 7, Figure A14.B) [55]. This was the only recipe where sodium (instead of calcium) carbonate was used. This is supported by the observation of characteristic bands of the $CO_3^{2-}$ ion at 1550 and 1409 $cm^{-1}$ (asymmetric stretching), 1100 $cm^{-1}$ (symmetric stretching) and 847 $cm^{-1}$ (bending). A band at 960 $cm^{-1}$ assigned to Al-OH bending is also present [55]. Moreover, the broadening of the band at 1100 $cm^{-1}$ indicates the presence of sodium sulphate, and the position of the band at 1409 $cm^{-1}$, slightly deviated from that expected, may also indicate the influence of a carbonate such as sodium or potassium carbonate.

The 19th century brazilwood reconstructions must be further investigated with different techniques in order to optimise the knowledge that can be gathered. However, the results presented are enough to make a comparison between 15th and 19th century brazilwood lake pigments.

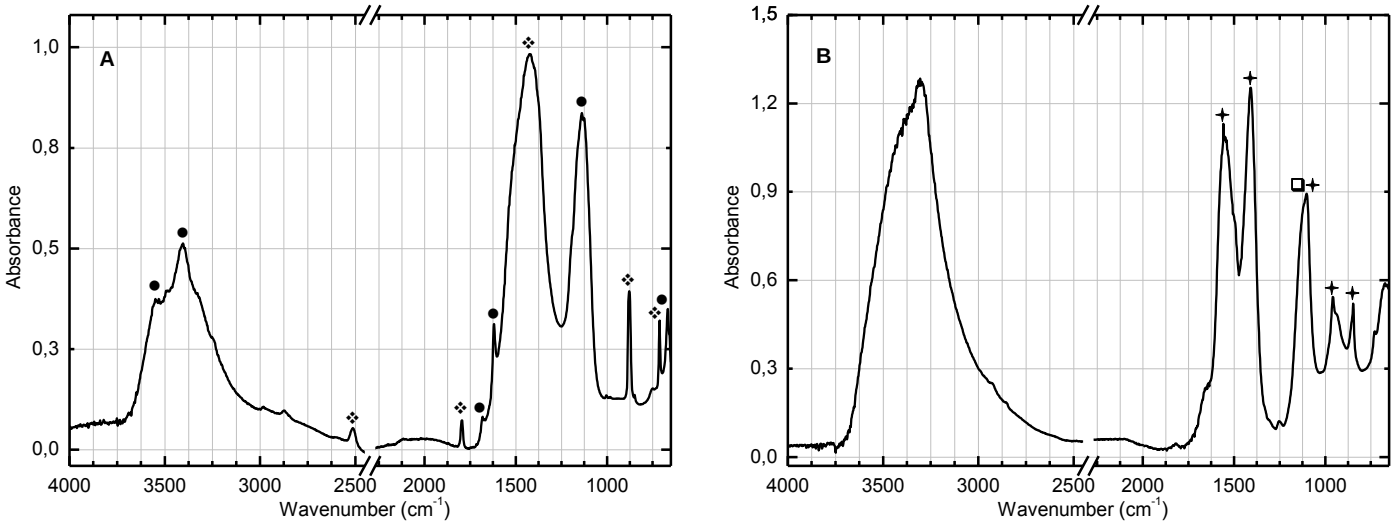


**Figure 17.** Infrared spectra of **A)** Rose Pink Seacy's (b) pigment and **B)** Wood Purple pigment; in which the main absorptions are assigned to: (❖) $CaCO_3$, (●) $CaSO_4 \cdot 2H_2O$, (✦) $NaAl(CO_3)(OH)_2$ and (❑) $Na_2SO_4$.

### 3.2.3. Comparing medieval and 19th century recipes

When comparing brazilwood lake pigments recipes from the *Livro de como se fazem as cores* and the W&N 19th century archive, some differences with respect to the information provided, the methods of extraction and precipitation, and the ingredients used can be noted. However, on the whole, the process to

prepare these pigments is similar (corresponding to that of lake pigments preparation) and, in the end, each difference is found to be present to give the same result (Table 6). Considering that both sources include aqueous extraction medium, $Al^{3+}$ as the mordant ion and ingredients that induce precipitation within a constant pH interval, it was possible to reproduce each recipe, using the same principles and compounds similar with respect to their action, and to obtain final pH values relevant to the pigment colour.

**Table 6.** Comparison between medieval and 19th century brazilwood lake pigments recipes.

| Source | Extraction method | Ingredients | Quantities | Alum : Brazilwood | pH of extraction‡ | Final pH‡ | Filtration |
|---|---|---|---|---|---|---|---|
| *Livro de como se fazem as cores* | Urine (neutral)<br>Lye, lime (basic) | Alum, chalk, gypsum, lead white | Seldom | 0.25 : 1 | 3.8 – 5.5<br>8.0 – 10.5 | 5.8 – 7.3 | Seldom |
| Winsor & Newton archive | Boiling water (slightly acidic) | Alum, chalk, cream of tartar, permuriate of tin(IV), potassium carbonate, carbonate of soda | Yes | 0.50 : 1<br>0.96 : 1 | 4.2 – 5.0 | 5.9 – 7.0 | Yes |

‡ Information obtained after recipes reproduction in the laboratory.

Considering the information given, in the medieval recipes it is, for whatever reason, often insufficient, subjective or unclear, whereas the 19th century records are more complete and present, in general, full information as stated above. Also, in the latter source commentaries on the final result obtained are also provided, which can be very useful in the recipes evaluation.

Possibly, the main difference in the production of the medieval and 19th century lake pigments is the pH of the dye extraction method. According to *Livro de como se fazem as cores* recipes, extraction can be performed in a basic (lye or lime) solution. As a consequence, the dye solution to which alum is then added has pH values between 8 and 10. In some cases, urine is used and alum is added during dye extraction yielding dye solutions with lower pH values. Dye extraction according to 19th century W&N recipes is carried out with boiling water and pH values of the dye solutions are between 4 and 5. Alum is only added afterwards. Nevertheless, due to the effect of the different ingredients later added, final pH values obtained with medieval and 19th century recipes are, in general, in the range that causes precipitation. It is interesting to note that the basic solution allowed for a fastest extraction of colour.

Concerning the ingredients, a difference can be noted with respect to the raw material. In medieval times only the species *C. sappan* was used as source for brazilwood dye. As such, in recipes from *Livro de como se fazem as cores* only the term “brazilwood” is present. Nonetheless, in the 19th century a variety of species associated to the name of the dye were recognised. Therefore, W&N recipes refer different names to indicate the brazilwood species used. With regard to pigment yield, considering the only medieval recipe that presents full information (chapter 8), it is fair to assume that, starting from the same brazilwood amount (5.0 g), the alum-dyestuff ratio is higher for W&N (e.g. 1.25 g is used for the medieval recipe, contrasting with 2.5 or 4.8 g used in the W&N recipes), which resulted in higher pigment yields (e.g. 45 % for chapter 9 and 24 % for chapter 44 *vs* 75 % for Rose Pink 1 and 63 % for Seacy’s Rose Pink).

Also, from the three extenders added during medieval pigment manufacture - chalk, gypsum and lead white -, only chalk can be found in the W&N recipes. Yet, gypsum was found to be formed during the reproduction of the latter when calcium carbonate is present. This may be explained by the higher content of alum (lower pH) added and it is only possible because $CO_3^{2-}$ from calcium carbonate is lost as $CO_2$[21]. In

[21] Otherwise, considering each compound solubility product constant ($K_{sp}$ ($CaCO_3$) = 3.36 x $10^{-9}$; $K_{sp}$ ($CaSO_4 \cdot 2H_2O$) = 3.14 x $10^{-5}$), $CaSO_4$ would not precipitate [3].

the 19th century recipes, sodium and potassium carbonate are added to increase the pH and act as buffers contributing to pigment precipitation. Cream of tartar is also a new entry although the purpose of its addition is not clear. Most interesting is the introduction of a tin salt together with alum in one of W&N recipes, providing the only aluminium- and tin-containing brazilwood pigment from both sources. This compound causes a very acidic environment but still precipitation occurs. The complex's molecular structure will probably be different and further analysis with NMR would be useful for its determination.

Considering the final pigments (painted with gum-arabic on parchment), hues obtained with brazilwood lake pigments can be very variable mainly with respect to the medieval case (Figure 18). As stated above, depending on the recipe from *Livro de como se fazem as cores* the following colours can be obtained: light and rosy pink, pink and red, and dark red. All 19th century W&N recipes yield rosy pink colours, rather similar between each other with the exception of Rose Pink, which presents very high L* values (Table 7). In general, 19th century colours have higher a* and b* values than those from medieval recipes.

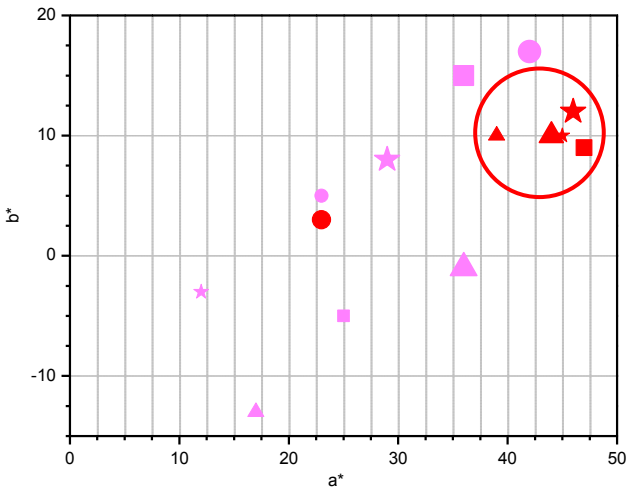


**Figure 18.** Representation of a* and b* coordinates of medieval and 19th century brazilwood lakes painted with gum-arabic on parchment (see Table 7).

| | L* | | b*/a* | |
|---|---|---|---|---|
| | min | max | min | max |
| Chapter 8 | 32 | 61 | ■ | ■ |
| Chapter 9 | 24 | 50 | ★ | ★ |
| Chapter 27 | 50 | 63 | ▲ | ▲ |
| Chapter 44 | 36 | 57 | ● | ● |
| Rose Pink | 81 | | ● | |
| Rose Pink II | 39 | 42 | ★ | ★ |
| Rose Pink Seacy | 61 | 68 | ▲ | ▲ |
| Wood Purple | 38 | | ■ | |

**Table 7.** L* values for medieval and 19th century brazilwood lakes painted with gum-arabic on parchment.

### 3.3. Comparison with Case-study

The pink and red colours found in the three French books of hours of the 15th century from *Palácio Nacional de Mafra* were analysed following the same multi-analytical approach carried out for the medieval brazilwood lake reconstructions. Results were compared with the latter so as to determine if it is possible to establish that the historical colours were prepared with brazilwood lakes and to better understand the technology of production of such colour materials.

In the three manuscripts (Appendix 11), the supposedly brazilwood colours are applied as light or dark opaque pinks, more or less translucent dark reds, glazes over gold and red, and to shadow blues and dark colours (Figure 19). Present in decorative flowers and borders, initials or in the central images, these hues are very similar to those from brazilwood reconstructions.

Ms 22 f76v Ms 23 f129r Ms 24 f53r Ms 24 f60r Ms 24 f116v

**Figure 19.** Details of pink and red colours found in the three French books of hours from PNM.

Gypsum, chalk, protein and polysaccharide binders were the main compounds detected by infrared spectroscopy. With respect to the latter, it was possible to clearly detect the presence of a protein such as egg white or parchment glue[22] due to the presence of the polyamide pattern at 1650, 1540 and 1450 $cm^{-1}$ [45]. A polysaccharide such as gum-arabic was also detected (bands at 1610, 1415 and in the region 1200 – 1000 $cm^{-1}$), in most of the cases together with the protein fingerprint [45]. The two binders may have been applied as a mixture or one of them may be present as a varnish. However, in some cases the presence of the polysaccharide cannot be completely confirmed due to the overlap of the absorptions from the extenders (Figure 20A). In which concern these white materials, in either red or pink colours gypsum (1134 and 670 $cm^{-1}$) is frequently found together with one or the two binders. Spectra revealing its presence together with the polysaccharide binder alone, such as that presented in Figure 20B, are the most common (present in 5 of the 8 folios analysed) and good matches with those from reconstructions were found. Sometimes calcium carbonate (1408, 875 and 713 $cm^{-1}$) is also present. Curiously, the latter was not found alone but always with gypsum. Possibly, chalk could have been converted into gypsum similarly to what was once observed with the medieval historically accurate reconstructions. Moreover, in manuscript 24, folio 60r two almost identical spectra revealing the present of the two white compounds were obtained for a pink and a dark red colour (Appendix 12, Figures A46.B and A47.A), the only difference being the proportion between gypsum and chalk. Even more interesting was the finding of a very small wood scrape over a black mantle from manuscript 24, folio 116v. Its infrared spectrum (Figure 21A) reveals the presence of the cellulose pattern (1160, 1115 and 1060 $cm^{-1}$) and other bands at 1560, 1500, 1460, 1428, 1375, 1327, 1240 and 900 $cm^{-1}$ that matches with the brazilwood spectrum (Appendix 7, Figure A21.A). Also, in a pink colour from manuscript 22, folio 76v, a similar spectrum was obtained and a good match with one medieval reconstruction painted with gum-arabic (chapter 8, experience 1, during which manufacture the filtration step was omitted) was obtained. Here, no binder could be clearly detected, although, in the case of the reconstructed pigment, a polysaccharide medium (gum-arabic) was used.

SEM-EDS analysis was performed in paint micro-samples from a pink decorative border from manuscript 22, folio 76v (Figure 21B) and a red colour from manuscript 23, folio 46r. In both, calcium carbonate and gypsum particles were detected. The former presents a rough appearance, irregular shape and large dimensions but is present in minor quantities. As such, this compound was not detected in the infrared analysis of the micro-sample from manuscript 22. On the other hand, gypsum particles present large dimensions and an irregular shape, uncharacteristic of the morphology of powder gypsum [3] yet similar to those of the brazilwood lake reconstruction also analysed with SEM-EDS (chapter 9, experience 5). In manuscript 23 micro-sample, it was also possible to identify the presence of small, irregular mercury and sulphur containing particles which can be associated with vermilion (mercuric sulphide, HgS). In the two micro-samples, the rough matrix area revealed the existence of high aluminium content indicating the presence of a dye-$Al^{3+}$ complex. However, especially in micro-sample from manuscript 22, this result contrasts with another matrix area which appears smoother and where very little aluminium is present. This difference may indicate the detection, in the latter area, of the binder used in the paint formulation that could also have been applied as a varnish. Infrared analysis of this micro-sample revealed the presence of both protein and polysaccharide binders. Finally, similarly to the results from the reconstructions, EDS analysis revealed the presence of Na, magnesium (Mg), Si, phosphorous (P) and chlorine (Cl) in minor quantities throughout the micro-samples (mainly in the rougher areas). These are most probably related to

[22] The distinction between the two protein binders with infrared analysis is not easy to do since both spectra are identical [45].

the natural dyestuff used during pigment manufacture [25]. Additionally, Ca, S and potassium (K) were also detected, most probably related to ingredients added during pigment manufacture.

Comparing SEM images of the historic paint micro-samples with those from paint reconstructions, it is clear that the latter appeared much more heterogeneous and seem to contain higher contents of extenders. However, in both cases, gypsum particles present an uncharacteristic morphology that may be related to the preparation of the pigments.

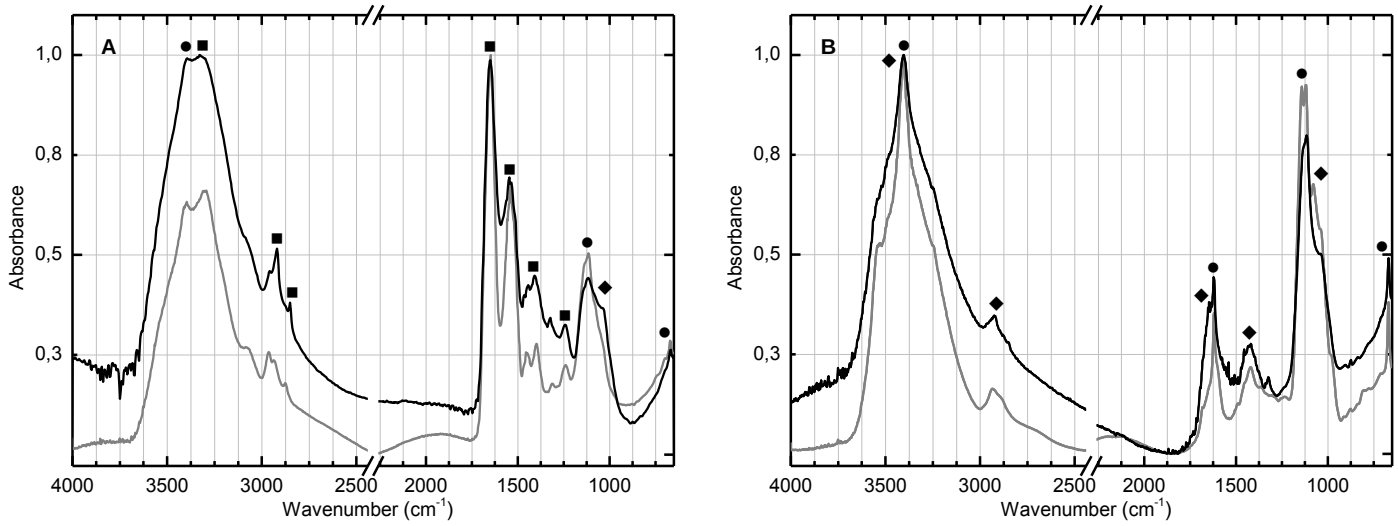


**Figure 20.** Infrared spectra of **A)** carmine decorative border µ-sample from Ms 22, folio 76v (**—**) and chapter 8, exp. 1 paint with egg white (—) and **B)** pink flower µ-sample from Ms 22, folio 76v (**—**) and chapter 9, exp. 5 paint with gum-arabic (—); in which the main absorptions are assigned to: (●) $CaSO_4 \cdot 2H_2O$, (◆) polysaccharide binder and (■) protein binder.

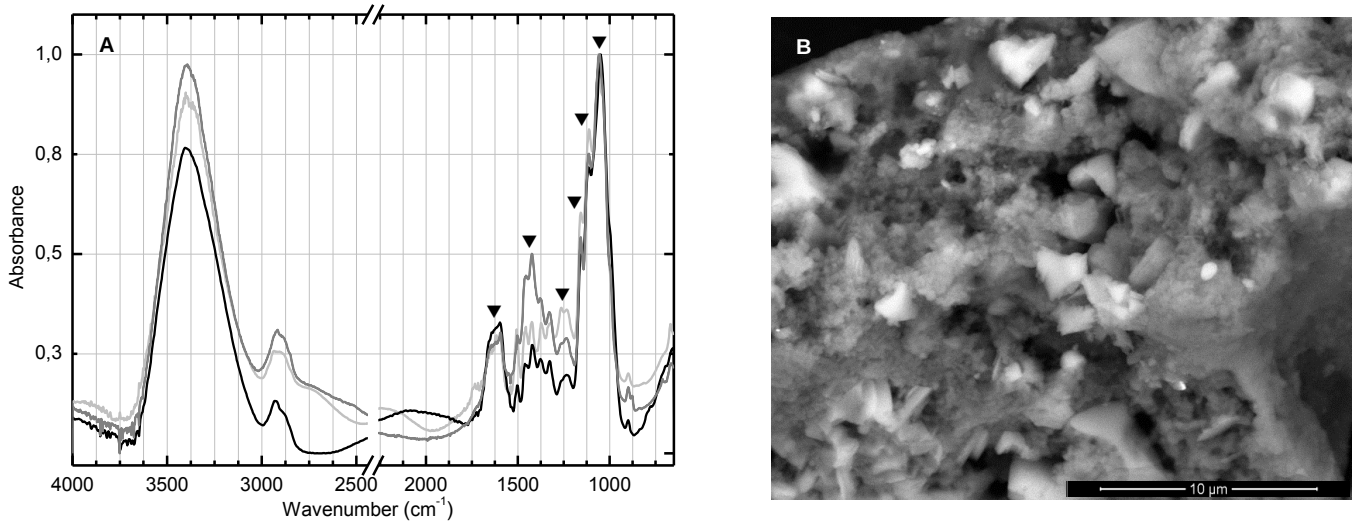


**Figure 21.** Infrared spectra of **A)** wood µ-sample from Ms 24, folio 116v (—), carmine glaze µ-sample from Ms 22, folio 76v (**—**) and chapter 8, exp. 1 paint with gum-arabic (—); in which the main absorptions are assigned to: (▼) cellulose; **B)** SEM image in BSE mode of carmine decorative border µ-sample from Ms 22, folio 76v.

Emission and excitation spectra were also acquired on pink and red micro-samples in order to obtain information on the dye. Good results were obtained and shape for both excitation and emission spectra proved to be similar in manuscripts 22 and 24, with maxima at *ca.* 560 and 585-90, respectively. The only difference was observed with the data obtained in manuscript 23, which present excitation $\lambda_{max}$ at 559-62 and emission $\lambda_{max}$ between 610 and 615 nm. Not only that, the excitation spectra present a slightly different

shape with a shoulder around 530 nm. This may be explained by a different chromophore environment or, most probably, by the presence of other emitters. Nonetheless, comparing the results from Ms 22 and 24 with the excitation and emission spectra of the brazilwood paint reconstructions a good match is obtained (Figure 22), indicating that the main chromophore present in the historical pink and red colours is possibly a brazilwood pigment.

### 3.3.1. *In situ* Analysis: μ-spectrofluorimetry *vs* FORS

The possibility to identify the dye with non-destructive, sensitive techniques, in which no micro-sample is required, was tested. FORS analysis was performed *in situ* on manuscripts 22 and 24 and excitation and emission spectra were acquired also *in situ* on manuscript 24.

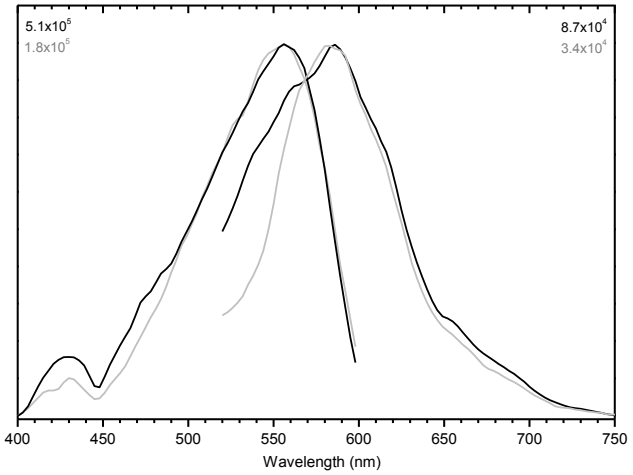


**Figure 22.** Emission and excitation spectra of paint μ-sample from Ms 22, folio 76v (—) and chapter 27, exp. 3 paint with glair on filter paper (—).

FORS spectral shape and absorption maxima (*ca.* 556 nm, Table 8) were remarkably consistent for the pink and red colours from the two manuscripts analysed with no visible influence of the paint substrate or binders as observed for the reconstructions. The only exceptions were observed for extremely light or saturated colours, in which less defined absorbance bands were obtained. Comparing the spectral data with that of reconstructions a good match is achieved (Figure 23A) indicating, once again, that the pink and red colours from the 15$^{th}$ century illuminations were most probably prepared with brazilwood lake pigments.

Emission and excitation spectra were also obtained *in situ* on manuscript 24 in order to compare the information acquired as such with that acquired with the micro-samples. Not only the *in situ* spectra proved to be reproducible with the latter but they also match with those of reconstructions (Figure 23B). Independently of the colour (pink or red) and binder, the emission and excitation spectra shape is similar and their maxima fall within the same range, that is, between 555 and 560 for the excitation, and between 583 and 592 for the emission analysis (Table 8). Slight differences between these values can be justified by possible different environments and/or the presence of other emitters. As previously referred, when comparing these results with those obtained for brazilwood reconstructions it is possible to propose that the historical colours - either pinks, dark reds or glazes - were prepared from such material.

The use of *in situ* techniques such as FORS and μ-spectrofluorimetry proved to be a valuable tool in analysing the illuminated manuscripts of the 15$^{th}$ century in a non-invasive way. Both approaches allowed for the acquisition of a high amount of data and for fast spectral acquisition with high spectral resolution and good S/N ratio. Moreover, the data obtained showed to be reliable and reproducible and match that of the reconstructions. Not only that, absorbance and excitation spectra proved to be very similar. Comparing the two techniques, FORS is a transportable technique, unlike μ-spectrofluorimetry, therefore does not necessarily require the removal of the manuscripts from their location. On the other hand, microspectrofluorimetry has the advantage of combining good spatial and spectral resolution with high sensitivity and selectivity, enabling the analysis of individual particles due to the use of a confocal microscope. Moreover, it allows for the simultaneous acquisition of excitation (which reproduces the

absorption of the molecule) and emission spectra [10]. Also, because different environments will provide different fluorescence spectra, it can give insight into the chromophore environment [45]. However, in this case, a full knowledge of the structure of the molecule and its behaviour is needed in order to optimise and enhance the potentialities of this technique.

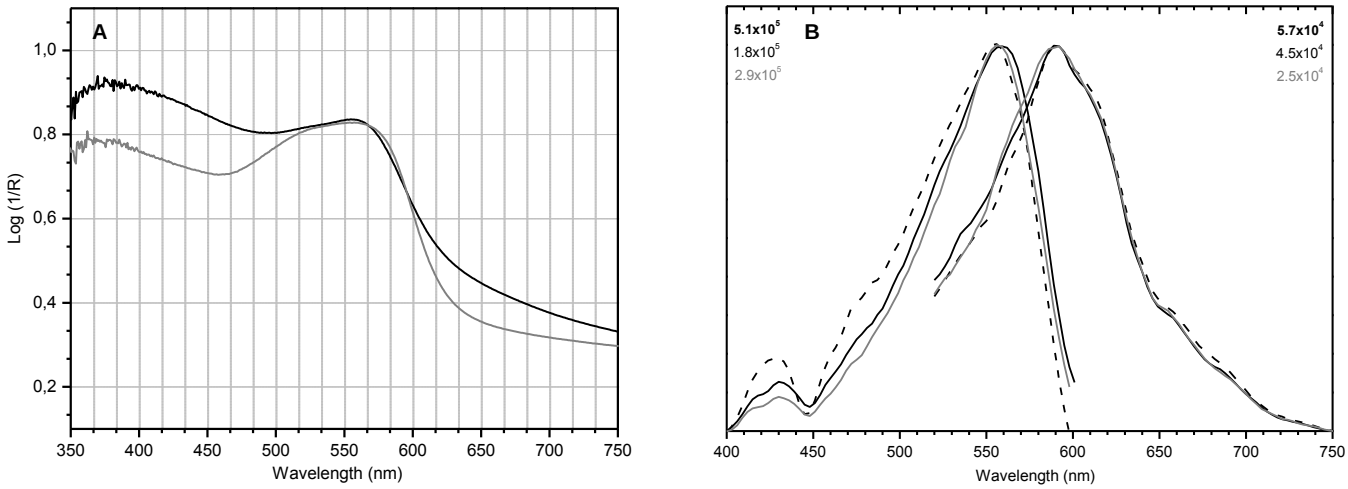


**Figure 23. A)** Absorbance spectra of carmine paint from Ms 22, folio 76v (**—**) and chapter 9, exp. 5 paint (—); **B)** Emission and excitation spectra of pink border from Ms 24, folio 60r: *in situ* analysis (**– –**) and µ-sample analysis (**—**) and of chapter 8, exp. 4 paint with glair on filter paper (—).

**Table 8.** Absorbance (FORS), excitation and emission maxima for the French books of hours from PNM.

| Ms | | 22 | | 23 | | | 24 | |
|---|---|---|---|---|---|---|---|---|
| folio | | 74r | 76v | 2r | 46r | 129r | 60r | 116v |
| **Absorbance λmax (nm)** | min | 552 | 556 | | | | 556 | 535 |
| | max | 556 | 557 | | | | 557 | 559 |
| **Excitation λmax (nm)** | min | | 556 | 562 | 560 | 562 | 555 | 560 |
| | max | | | | | | 557 | |
| **Emission λmax (nm)** | min | | 585 | 610 | 610 | 615 | 583 | 586 |
| | max | | | | 615 | | 590 | 592 |

## 4. Conclusions

The study of brazilwood and its lake pigments based on the characterisation of a pure brazilein reference and on historically accurate reconstructions, using a multi-analytical approach, was carried out. Most importantly, it was possible to verify that the 15$^{th}$ century *Livro de como se fazem as cores*, so distant in time, allowed for the successful preparation of brazilwood lake pigments with a variety of colours and that the manufacture of these pigments was fairly maintained until the 19$^{th}$ century. Furthermore, infrared spectroscopy, on the one hand, and reflectance and fluorescence spectroscopy, on the other hand, proved to be three essential and complementary techniques to the characterisation of the paints produced.

The preparation of the reconstructions based on recipes from the 15$^{th}$ century documentary text *Livro de como se fazem as cores* and the Winsor & Newton 19$^{th}$ century archive, both valuable and representative sources of their time, was essential for insight into what methods and materials were used in the past to create brazilwood lake pigments. Despite some differences were noticed between the two sources, mainly with respect to the dye extraction method, both are guided by the same principles and the

final pH obtained is always adequate for pigment precipitation. Some different ingredients are present but to produce the same effect. Alum and chalk are two ingredients that are maintained, while gypsum can be found only in the medieval recipes and the 19$^{th}$ century source presents a new range of compounds. Curiously, in the latter, tin is added as a precipitating agent, together with aluminium, allowing pigment precipitation at very acidic conditions. Brazilwood lake pigments can be obtained in a variety of different hues between light pink and dark red as was observed by colorimetry results. However, a note should be made to the fact that, after extraction, much colour still remains in the brazilwood raw material.

Pigments and paints further characterisation provided reference samples and new knowledge to its study in works of art. Infrared analysis was essential to characterise the extenders. Gypsum was found to occur as a by-product of manufacture, formed during pigment synthesis by the simultaneous presence of $SO_4^{2-}$ ions from alum and $Ca^{2+}$ from calcium carbonate in solution, at acidic conditions. This situation was observed mainly in the 19$^{th}$ century case where alum is added in higher quantities, and is of particular importance for the analysis of paints on artworks. Also interesting was the formation of dawsonite during the synthesis of a pigment based on a sodium carbonate-containing 19$^{th}$ recipe. As such, the presence of this compound may be related to the use of sodium instead of calcium carbonate, together with alum, in the recipes. Infrared spectra of paints showed that the pigment signature can be totally or partially masked by the binder, either gum-arabic or egg white. SEM-EDS was also useful to give information on extenders particles morphology, the homogeneity of the paints, and the lake mordant by the detection of $Al^{3+}$.

FORS and µ-spectrofluorimetry were important to identify the chromophore. FORS data of the various hues prepared with brazilwood were remarkably consistent in shape and absorption maxima (560 nm). Fluorescence spectra present $\lambda_{max}$ at *ca.* 553 nm for excitation and 585-90 nm for emission. For both techniques, the different paints' substrate, recipes, extenders or binders had no influence on the spectra, and no discrimination could be made between the different colours that were obtained with brazilwood. In other words, FORS and fluorescence spectra proved to be very characteristic of the brazilwood pigment.

Medieval reconstructions were compared with red and pink colours of illuminations found in three French books of hours (PNM, Portugal) from the 15$^{th}$ century. Good correlation between FTIR, FORS and µ-spectrofluorimetry results of the reconstructions and the case study suggest that the illuminations colours were prepared with brazilwood lakes. The similarity found between the colours produced based on the 15$^{th}$ century recipes and those from the books of hours offers encouraging evidence that the colours in the manuscripts' illumination have not changed significantly. This shows that despite their reputation for being relatively unstable and subject to fading, the brazilwood colours in the books of hours are very well preserved. Moreover, the use of FORS and µ-spectrofluorimetry as *in situ* techniques proved to be a valuable tool in analysing works of art in a non-invasive way, allowing for fast spectral acquisition with high spectral resolution and good S/N ratio. Also, the data obtained are reliable and reproducible.

Still and all, a full knowledge of the structure of the molecules analysed and their behaviour, as well as the creation of an organic dyes database, are extremely important and will help to optimise and enhance the potentialities of both techniques. In particular, cochineal lake pigments and a mixture of the latter with brazilwood must be reproduced and characterised to better discriminate between the results obtained.

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## Appendices

**APPENDIX 1.** Transcription of brazilwood lake pigments recipes

## 1.1. Transcription of *Livro de como se fazem as cores* recipes[i]

### Chapter 8

**Transliteration**

**kapitulo 8** pera fazer ros$^{a}$h | toma uah onç$^{a}$h | de brasil fino e rapa-o meudo e pon-o | adeparte e depois toma uah kuart$^{a}$h | onça de pedra ume e toma peso de dos | dineros de alvai alde e moy-o kon a.pedra ume | en un almofaris e pon-o aparte e toma | depois o brasil e deita-o en uah | taç$^{a}$h de maleg$^{a}$h e deit$^{a}$h os otros pous | e kon o brasil e deita-ly$^{e}$ e çima urin$^{a}$h | ata ke se reskobrao e esten asi per 3 | dias akabados e toda viah meçendo-os | kon un pao k$^{a}$da diah 5 o 6 vezes. e despois | koa-o e apora-o por un pano de | linyo e çima de uah piah feit$^{a}$h de gis o de pedra kri. e lesa-o bibir na piah e kuando for ensuto rapa-o muy | bein kon uah paleit$^{a}$h. e guarda-o bein do | ayre e kuando kiseires la*b*rar ko.ele | moio kon aguah gomada

**English translation**

**Chapter 8.** In order to make rose, take one ounce of fine brazil-wood, and scrape it very fine, and set it aside. And then take a quarter ounce of alum and take two pennyweights of white lead and grind it with the alum in a mortar and set it aside. And then take the brazil-wood and place it in a cup [made] of *malega*, and put in the other powders with the brazil-wood, and pour urine over them until they are covered. And let them stand thus three whole days, always stirring them with a stick 5 or 6 times each day. And then pass it and strain it through a linen cloth above a trough made of gypsum or chalk-stone. And let it soak in the trough, and when it is dry, scrape it very well with a spatula, and keep it carefully from the air. And when you want to work with it grind it with gum water.

### Chapter 9

**Transliteration**

**kapitulo 9** | pera fazer outra ros$^{a}$h | toma do brasil oke uberes mester e | ahrapa o been meudo e deita-o en un$^{a}$h | olya pekena noba e deit$^{a}$h na olya de koad$^{a}$h | de vides ke seg$^{a}$h o brasil dela koberto | poen-$^{a}$h ao fogo e da-ly$^{e}$ uah | fer vora atanto ke tome adekuada sustançiah | do brasil e toma duas p$^{a}$rtes | de pedra ume e mais a.meiah parte de | pedra kri e moy$^{e}$ kada un muyto por si e | depois mistura-o e moya de konson e faze | komo ga sabes de pedra ume ros$^{a}$h

**English translation**

**Chapter 9.** In order to make another rose colour, take brazil wood, as much as you need, and scrape it very fine and place it in a small new pot. And place in the pot lye of vine branches, so that the brazil wood is covered with it. Put it on the fire and heat it enough for the lye to take up the substance of the brazil wood. And take two part of alum and at least a half a part of chalk and grind each one well by itself. And then mix it and grind it together, and make, as you already know, rose out of alum

[i] a) Strolovitch, D. 2010. O libro de komo se fazen as cores das tintas todas (Transliteration). In: Afonso, L. ed. *As Matérias da Imagem*, Volume 3. Lisboa: Cátedra de Estudos Sefarditas «Alberto Benveniste», pp. 213-223. b) Strolovitch, D. 2010. O libro de komo se fazen as cores das tintas todas (Translation). In: Afonso, L. ed. *As Matérias da Imagem*, Volume 3. Lisboa: Cátedra de Estudos Sefarditas «Alberto Benveniste», pp. 225-236.

**Chapter 27**

**Transliteration**

**kapitulo 27** si kiseres | fazer boah ros$^{a}$h filya | do brasil kuanto kiseres e rapa o muy ben | ençima de uah kong$^{a}$h o korno e desae | agunta kon el da pedra ume e deske esto | fizeres filya da urina do omen kasto e | deita tanta ençima dese brasil e da pedra | ume ata ke sega tres kobertos e lesa-os | asi estar por 3 dias e depois filya un | pao de gis e deita do pou dele ençima dese | brasil ata ke se mely$^{e}$ ke seg$^{a}$h tanto doun komo dotro e desae leisa-o asi estar esa | konfaçion por un diah e por dois e depois | toma esa rosa e moya kon krara do*b*o gomada | e eskrebe ko.el$^{a}$h

si kigeres fazer | fazer koor indiah | mete kon el do azul e si por ventura kiseres | fazer *pinça* negra mete kon el do negro e si | por ventura akor alva kiseres tornar en | negro agunta kon el do negro e do alvo e toma | do brasil e mete-o en un pano alvo e koa-o | sobre gis. sabe ke des sao as kores prinçipais | azul. oripimento e vermelyon. | verde. karmen. çuf$^{i}$y. katasol. açafrao. | az$^{a}$rkon. al*v*ay$^{a}$lde. brasil. kuando kiseres | kebrar la krara do o*b*o ega aly$^{e}$ | dela lege dela figeira e kebralya as muito bein | para tu obra klara komo aguah

**English translation**

**Chapter 27.** If you wish to make good rose-colour, take brazil-wood, as much as you want, and scrape it well over a conch or retort, then add alum to it. And once you have done this, take the urine of a chaste man and add pour it over the brazil-wood and the alum until they are well covered, and let them stand thus for three days. And then take a piece of chalk and throw some of its powder over this brazil-wood until it seems that there is as much of one as of the other. And then let this concoction stand for a day or two. And then take this rose-colour and grind it with gummed egg-white, and write with it.

If you wish to make indigo, put blue in it, and if perhaps you wish to make a black color, put black in it. And if perhaps you wish to turn white color black, add black to it and white and take brazil-wood and place it in a white cloth and strain it over chalk. Know that the principal colors are ten: blue, oripiment, and vermilion, green, Sufi carmine, sunflower, saffron, red lead, white lead, brazil-wood. When you want to thin egg-white add the sap of a fig tree to it, and thin it very well, clear as water, for your work.

**Chapter 44**

**Transliteration**

**kapitulo 44** si kiseres fazer | boah roseta toma o | brasil e mole-o no almofaris ke sega been moído | peny$^{e}$rao e toma uah pok$^{a}$h de kal virgen e | pona nuah altamiah kon aguah ates ke se faça | a aguah krar$^{a}$h e kon akel$^{a}$h aguah moy o brasil e lançaly$^{e}$ un pekeno de pedra ume e destenpera | kon goma e eskrebe kon el

**English translation**

**Chapter 44.** If you wish to make good rose-colour, take brazil-wood and grind it in a mortar, until it is well ground. Sift it, and take a little virgin lime and place it in a glazed earthenware bowl with water until the water becomes clear, and with this water grind the brazil-wood, and put in it a little alum, temper it with gum, and write with it.

## 1.2. Transcription of Winsor & Newton 19th century archive recipes

**Recipe Name Original:** Rose Pink **Unique Recipe Code:** P2P012AL01 **Recipe Date:** (-)

Rose Pink

1237. Take 2½ lbs brazilletta (7 cwt)

½ lbs west India Peach wood (13 cwt) in 350 gall copper pan. Boil in water 2½ hours, then add 1 lb cream tartar (turned yellower) after strained, add 1 cwt pow$^{d}$ alum. Add 2½ cwt whiting.

Brown Holland bag for filters, box with holes.

Turns out full 3½ cwt Rose Pink. The alum occasions a precipitate in the liquor of itself.

**Recipe Name Original:** Rose Pink[ii] **Unique Recipe Code:** P2P012BL01 **Recipe Date:** (-)

Rose Pink. Mem: from Simmonds

1237A. 300 lbs j(s)apan wood

148 lbs alum

264 lbs whiting

**Recipe Name Original:** Rose Pink. **Unique Recipe Code:** P2P012BL05 **Recipe Date:** 05-1849

Searcy's Formula

Rose Pink. Seacy's formula mar 1849

1237B. 10½ lbs wood (nigeraga wood I believe) boiled in about 16 galls water 3 hours frequently stirred / strain. Serve it twice more in same manner to draw out all the colour. Strain the coloured liquor into the other (viz the liquor of the 3 boilings all put together). Then add 10 lbs ground alum diss$^{d}$ in hot water. Then add to the coloured liquor about 10 lbs of a solution of permuriate of tin (or enough to precipitate nearly all the colouring matter). Then add about ¼ lb of pearlash diss$^{d}$ in 2 galls hot water. This should complete the precip$^{n}$ of the colour and the water should be nearly clean. The precipitated colour should now be well washed.

Prepare the body as follows: Take 30 lbs alum dissolve in boiling water (about 20 galls). Take 40 lbs whiting break it up into a thinnish paste with boiling water and pour the hot solution of alum over it by a pailful or two at a time and stir thoroughly well. An effervescence takes place. When all the alum is in / the body so made must be 2 or 3 times washed and passed thro' a hair sieve.

Mem$^{o}$ the solution of tin should be prep$^{d}$ by dissolving tin in a mixture of 1 part sol(?) acid and 3 parts muriatic acid (with water sufficient) done by degrees. Takes all day.

Seacy says that some houses make a strong solution of extract and throw it upon whiting which takes it up but it is not so fine.

**Recipe Name Original:** Wood Purple **Unique Recipe Code:** P2P021AL01 **Recipe Date:** 05-03-1849

Wood Purple – Seacy's method Mar 5/49

1264. Take 3½ lbs lima peachwood boiled in about 6 galls water. Gave a strong solution if to this be added (after straining) about 15 galls more cold aq and about 6 lbs carb$^{te}$ soda and then precip with 6 lbs pow$^{d}$ alum strewed in. It gives a good sort of dull rich crimson. Seacy says that less than 6 lbs each of soda and alum would not take down all the colour – this seemed rather specky or fluffly in grinding. Colour very rich and transparent. More so than from cochineal.

Produce 1 Lb 9 oz. Fades much quicker than cochineal.

[ii] Here distinguished by the name "Rose Pink II".

**APPENDIX 2.** Experimental section

## 2.1. Materials

All reagents used were of analytical grade. Spectroscopic or equivalent grade solvents and Millipore filtered water were used for all the chromatography and spectroscopy studies and further experimental work. Spectroscopy studies in the liquid state were carried out in quartz cells; for the solid state qualitative filter paper from Filter Lab was used.

For both brazilein isolation and pigments reconstructions, *Caesalpinia echinata* from Kremer was used. *Caesalpinia sappan* and *Haematoxylum brasiletto* were kindly provided by the Waimea Valley (Hawaii, USA) and Boyce Thompson Arboretum (Arizona, USA). *Haematoxylum campechianum* from Kremer was also used. For the preparation of medieval and 19$^{th}$ century historically accurate reconstructions aluminium potassium sulphate dodecahydrate ($AlK(SO_4)_2 \cdot 12H_2O$), sodium carbonate ($Na_2CO_3$) and potassium carbonate ($K_2CO_3$) from Riedel-de Haën; sodium hydroxide (NaOH) from Eka Chemicals; calcium carbonate ($CaCO_3$) from Roig Farma; tin(IV) chloride pentahydrate ($SnCl_4 \cdot 5H_2O$) from Sigma-Aldrich; potassium hydrogen tartrate ($C_4H_5KO_6$) from Scharlau; and calcium sulphate dihydrate ($CaSO_4 \cdot 2H_2O$) from Panreac were used. Lye and lime solutions with pH values between 11 and 12 were prepared with wood ashes and calcium oxide (CaO) from Acrilar, respectively. Fresh urine from a one individual with pH around 7 was used. Basic lead carbonate ($2PbCO_3.Pb(OH)_2$) prepared according to the "Dutch" (or "stack") process and a calcium sulphate dihydrate bowl offered by Robert Wiley from Vicarte (FCT/UNL) were also used. For infrared reference spectrum aluminium hydroxide ($Al(OH)_3$) from Sigma-Aldrich was used. Gum-arabic from Bizzarri, fresh eggs and natural parchment from Musée du Parchemin were the raw material for binders preparation. Glass recipients and tools were used during all reconstructions in an effort to minimize contamination.

## 2.2. Experimental methods

### *Brazilein isolation*

Brazilein was extracted from brazilwood chips according to the method described in Berger & Sicker (2009) and with the help of Professor Jorge Parola. 300g of chips were allowed to stand in 1700 mL methanol for extraction during three days. The mixture was filtrated and the filtrated solution was concentrated at a rotary evaporator (bath temperature 45°C) to a volume of 100 mL which was allowed to stand for four days. The black crystals of brazilein that precipitated were filtered off by suction, washed with ice-cold methanol and dried in vacuum. The latter procedure was repeated twice more yielding a total of 900 mg of brazilein (230 + 630 mg obtained from first and second precipitations, respectively).

### *Brazilein characterisation*

Nuclear Magnetic Resonance spectroscopy ($H^1NMR$) and High Performance Liquid Chromatography equipped with Diode Array Detector (HPLC-DAD) were used to confirm brazilein's molecular structure and purity.

UV-Vis pH titrations were performed in a $10^{-4}$ M brazilein mixture of methanol and water (70:30, v/v) and of water and methanol (70:30, v/v) to investigate if the molecule's behaviour would be different depending on the water - methanol ratio. To both mixtures, solutions of HCl and NaOH with different concentrations were added. The behaviour of $10^{-4}$ M brazilein in methanol and in the mixture of water and

methanol (70:30, v/v) at different pH's throughout time was also studied by UV-Vis spectroscopy. The different pH's (pH 2.9 and 6.1) were obtained through the addition of HCl and NaOH solutions to the mixture of water and methanol. A dye-$Al^{3+}$ complex was produced through the addition of $AlCl_3$ (aluminium chloride) to a $10^{-4}$ M brazilein mixture of water and methanol (70:30, v/v) and its absorption properties were characterised with UV-Vis spectroscopy. A fluorescence $Al^{3+}$ titration was performed using a $10^{-5}$ M brazilein solution in a mixture of water and methanol (70:30, v/v) to which $AlCl_3$ with different concentrations was added in order to obtain different $Al^{3+}$-dye ratios (x 2; x 5; x 10; x 50; x 100; x 1000).

Brazilein characterisation at different pH's as well as that of brazilein-$Al^{3+}$ complex was also made in the solid state (filter paper) by fluorescence and UV-Vis spectroscopy. For this, four brazilein $10^{-3}$ M solutions were used: in methanol; in methanol with the addition of $AlCl_3$; in water and methanol (70:30, v/v) with the addition of HCl (to obtain a pH of 2); and in water and methanol (70:30, v/v) with the addition of NaOH (to obtain a pH of 8). Three drops of each solution were then applied on filter paper.

Part of the work related to brazilein's characterisation was also carried out with the help of Professor Jorge Parola.

***HPLC-DAD preparation***

For the characterisation of the brazilwood red chromophore, the isolated brazilein was prepared as an $8.0 \times 10^{-4}$ M solution in methanol. A few µL of perchloric acid were added to keep it at a low pH value during the analysis. The solution was then dissolved in 30% water and injected onto the HPLC-DAD system.

To characterise the four wood species, they were left to stand in boiling water for three hours. The solutions were filtered and methanol was added in order to obtain water:methanol (50:50, v/v) solutions. A few µL of perchloric acid were added to keep them at a low pH value during the analysis and each solution was injected onto the HPLC-DAD system.

For the extraction of medieval brazilwood lake pigments reconstructions, a mild extraction method (oxalic acid) was used. 0.2–0.4 mg of each pigment were weighted and heated at 60ºC in 400 µL of a 0.2M oxalic acid/methanol/acetone/water, 0.1:3:3:4 (v/v/v/v) solution for 30 minutes, always under constant mechanical agitation. The solutions were filtrated and left to concentrate. 50 µL of a mixture of methanol:water (70:30, v/v) were added and the solutions were centrifuged for 10 minutes. The upper 40 µL of each solution was removed and injected onto the HPLC-DAD system.

***Binders preparation***

A 10% gum-arabic solution was prepared as follows: the gum-arabic was ground and left to stand in water for one day; it was then filtrated and the filtrated solution was kept as the binder. Egg white was prepared by beating the egg white and leaving it to stand for some hours. The liquid obtained was also kept as the binder. Parchment glue was prepared by boiling parchment pieces in water until it is good to act as glue. The pieces of parchment are then put aside and the solution is kept. All three binders were stored in the refrigerator.

Besides these three binders used with the reconstructions (chosen to represent typical binders for illuminated manuscripts), those that could eventually appear in case-study micro-samples (mixture of gum-arabic and egg white (3:1) and transparent starch solution) were also prepared and analysed by infrared spectroscopy.

### 2.3. Apparatus

SEM-EDS analysis was performed within the Portuguese microscopy network REM, at CEMUP-Centro de Materiais University of Porto, by Rui Rocha. FORS analysis was carried out *in situ* by Marcello Picollo. $H^1$NMR analysis was performed within the NMR national network (supported with funds from FCT-MCTES, Portugal), at the LabRMN, FCT-UNL. All the other analyses were carried out at the DCR-FCT/UNL.

The author of this work is grateful to Ana Marta Diniz, Jorge Parola, Cristina Montagner, Andreia Ruivo and Augusta Lima for their support with respect to some apparatus.

***$H^1$NMR***

Brazilein was dissolved in $(CD_3)_2SO$. $H^1$NMR spectrum was run on a Bruker AMX 400 instrument operating at 400.13 MHz.

***HPLC-DAD***

The analyses were carried out in an analytical Thermo Electron, Finnigan™ Surveyor® HPLC-DAD system with a Thermo Electron, Finnigan™ Surveyor® LC Pump, Autosampler and PDA Detector, and using a reversed-phase RP18 analytic column (Nucleosil C18, 250 x 4.6 mm, 300 Å - 5 µm) kept at controlled temperature (35 ºC). In the analytical system the samples were injected onto the column via a Rheodyne injector with a 25 µL loop. The elution gradient used at a flow rate of 1.7 mL/min consisted of A: HPLC-grade methanol and B: 0.3% (v/v) acid perchloric in Millipore water. The gradient elution program was: for 0-2 min, isocratic 7% A; for 2-8 min, linear gradient to 15% A; for 8-25 min, linear gradient to 75% A; for 25-27 min, linear gradient to 80% A; for 27-29 min, linear to 100% A; and for 29-40 min, isocratic 100% A.

***Infrared spectroscopy***

FTIR analyses were carried out using a Nicolet Nexus spectrophotometer coupled to a Continuµm microscope (15x objective) with a MCT-A detector cooled by liquid nitrogen. Spectra were obtained in transmission mode, between 4000 – 650 $cm^{-1}$, with a resolution of 4 $cm^{-1}$ and 128 or 256 scans. Samples were previously compressed using a Thermo diamond anvil compression cell. Spectra are shown here as acquired, without corrections or any further manipulations, except for the occasional removal of the $CO_2$ absorption at *ca.* 2300–2400 $cm^{-1}$.

In a few cases, FTIR analyses were performed on the same Nicolet Nexus spectrophotometer using KBr pellets. The spectra were collected in transmission mode, between 4000 – 400 $cm^{-1}$, with a resolution of 4 $cm^{-1}$ and 64 scans. Preparation of KBr pellets involved mixing and grinding a small quantity of the sample with KBr powder and then pressing it under high pressure (± 8.5 tons).

***SEM-EDS***

SEM images were obtained using a FEI Quanta 400 FEG ESEM, which uses a Schottky emitter field emission gun, operating at low vacuum conditions and at 15 kV, equipped with an EDAX Genesis X4M detector. Images were acquired using secondary (SE) and backscattered (BSE) electron detectors.

***Micro-spectrofluorimetry***

Fluorescence spectra were recorded on a Jovin-Yvon SPEX Fluorog 3-2.2 spectrofluorometer. Fluorescence spectra were corrected for the wavelength response of the system. For µ-spectrofluorimetry

analyses, a micro-Spex (Spex® FluoroMap with Manual Microscope Stage) was used, where the SPEX Fluorog 3-2.2 equipment was hyphened to an Olympus BX51 M confocal microscope, with spatial resolution controlled with a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot with 50x objective. A continuous 450 W Xenon lamp, providing an intense broad spectrum from the UV to near-IR was used. Standard dichroic filters of 500 and 600 nm were used at 45° to collect the emission and excitation spectra, respectively. Emission spectra were acquired exciting at 490 nm and excitation spectra were performed collecting the signal at 610 or 620 nm. Both were acquired in a 30 μm spot (pinhole 8) and the following slits set: emission slits = 3 / 3 / 3 mm, and excitation slits = 5 / 3 / 0.8 mm. A spectral resolution of 2 nm was always used. The optimization of the signal, through mirror alignment in the optic pathway of the microscope, was performed, for all pinhole apertures, following the manufacturer instructions. Moreover, spectra were collected after focusing on the sample (eye view) followed by signal intensity optimization (detector reading). Emission and excitation spectra were acquired in the same spot whenever possible.

***UV-Vis spectroscopy***

FORS measurements were performed using two Zeiss spectroanalysers equipped with optical fibres: a MCS 601 UV/VIS model (with a 1024 Si photodiode array sensor) operating in the 190-1025 nm range, and a MCS 611 NIR 2.2 WR model (with a 256 InGaAs photodiode array detector) operating in the 910-2200 nm range, with a resolution of approximately 0.8 and 5.0 nm/pixel, respectively. A tungsten-halogen lamp (Zeiss model CLH600) was used. The 0°/2x45° reflectance configuration was adopted to avoid specular reflectance. A 99% Spectralon diffuse reflectance standard was used for calibration. In each analysis, at least three different areas for pink and red colours were analysed so the data may be representative. FORS spectra were acquired in reflectance but are presented as apparent absorbance, A' = $Log_{10}(1/R)$.

Reflectance spectra were also acquired using an UV-Vis portable fiber optic spectrometer Avantes AvaSpec-2048 with a 300 lines/mm grating. The operational range is 200-1100 nm and the instrument has an FWHM resolution of 2.4 nm. An Avalight-Hal Tungsten Halogen Light source and an Avasphere - 50 - REFL integrating sphere were used. Reflectance spectra are presented as apparent absorbance, A' = $Log_{10}(1/R)$.

Absorption spectra in transmittance were recorded on a Shimadzu UV-250 IPC Spectrophotometer.

Lab* coordinates were measured with a portable spectrophotometer colorimetry Data Color International. The CIELAB system was used, defining the D65 illuminant and the 10º observer. Calibration was performed with white (porcelain) and black prototypes.

***pH measurements***

pH measurements were made with a Sartorius Docu-pH Meter. Calibration was performed with pH 4 and 7 buffer solutions (Panreac).

**APPENDIX 3.** Characterisation of brazilein: $H^1$NMR, UV-Vis spectroscopy and Spectrofluorimetry

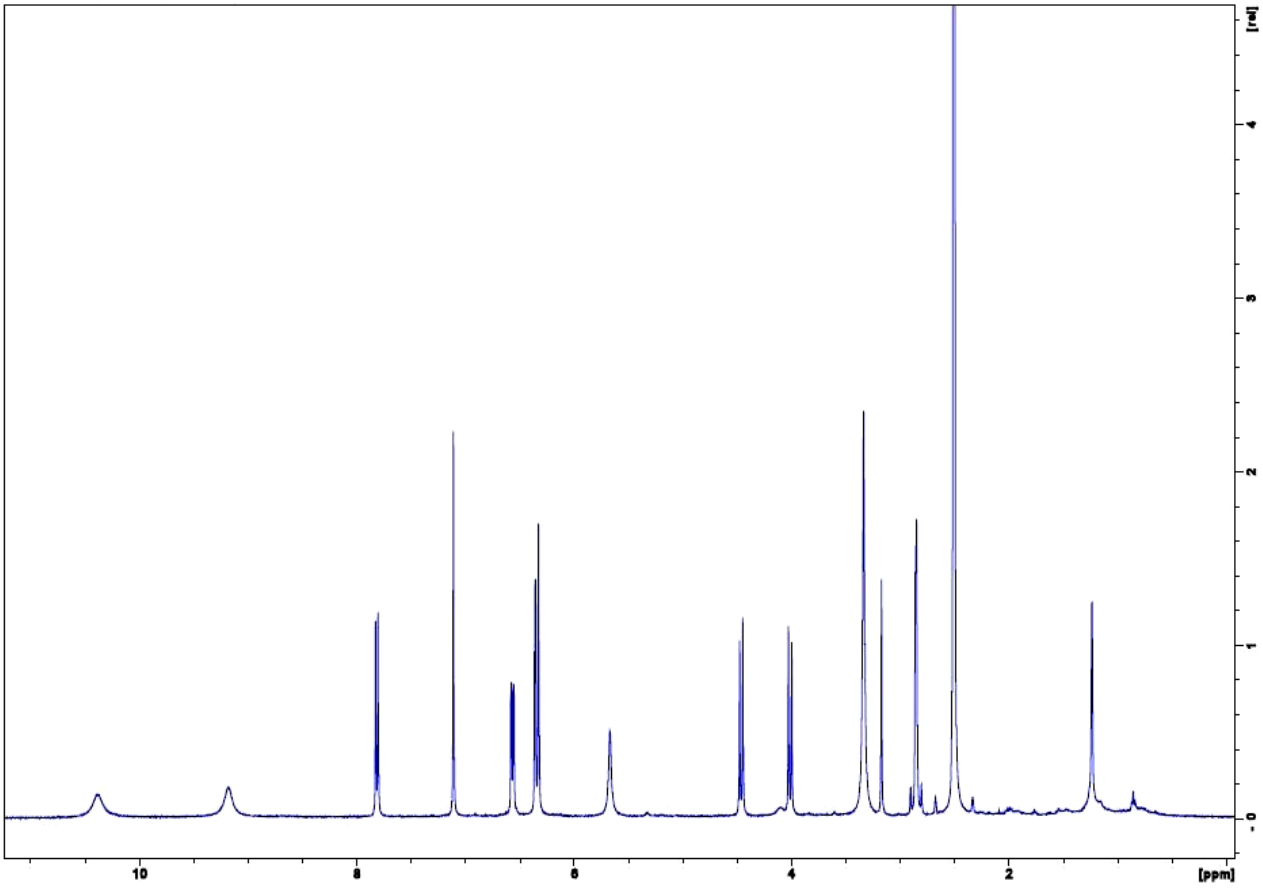


**Figure A1.** $^1$H NMR spectrum at 400 MHz in DMSO-$d_6$ of brazilein isolated from *Caesalpinia echinata*.

The proton NMR spectrum, recorded in DMSO is rather similar to that presented in Berger and Sicker (2009), where a complete analysis - which is outside the scope of this work - can be found. A single note should be made to the signal at 3.3 ppm that corresponds to the residual water in DMSO.

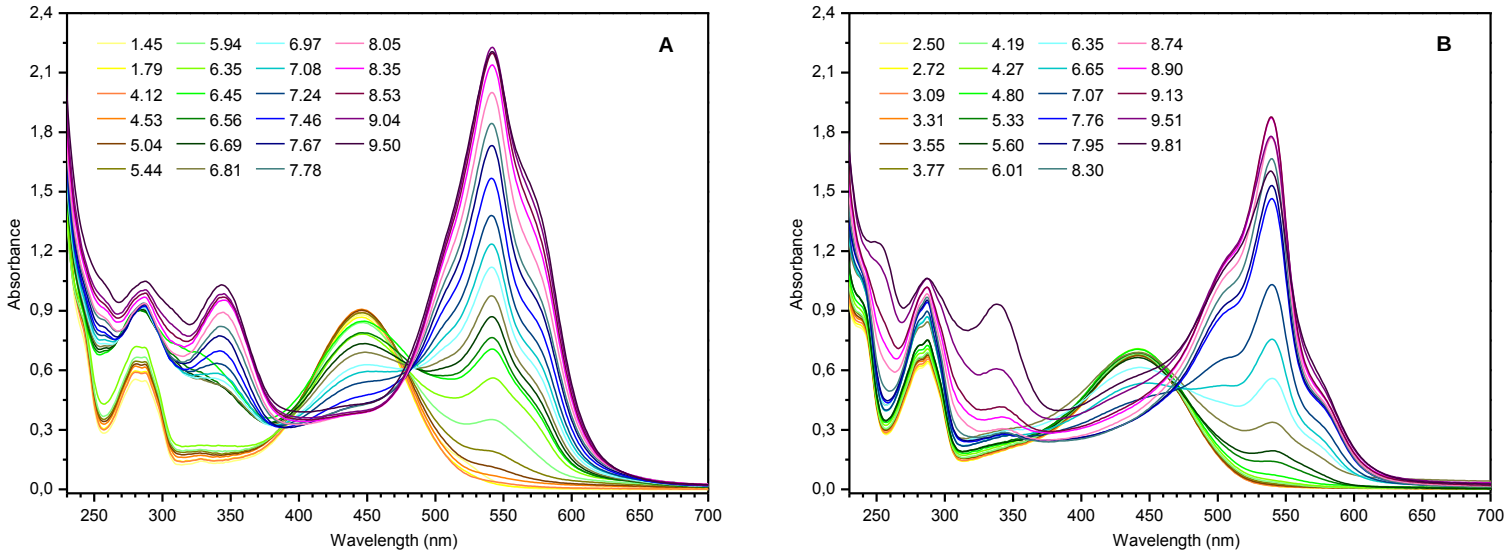


**Figure A2.** UV-Vis pH titration of brazilein $10^{-4}$ M in **A)** MeOH:$H_2O$ (70:30, v/v) and **B)** $H_2O$:MeOH (70:30, v/v); with corrected absorbance.

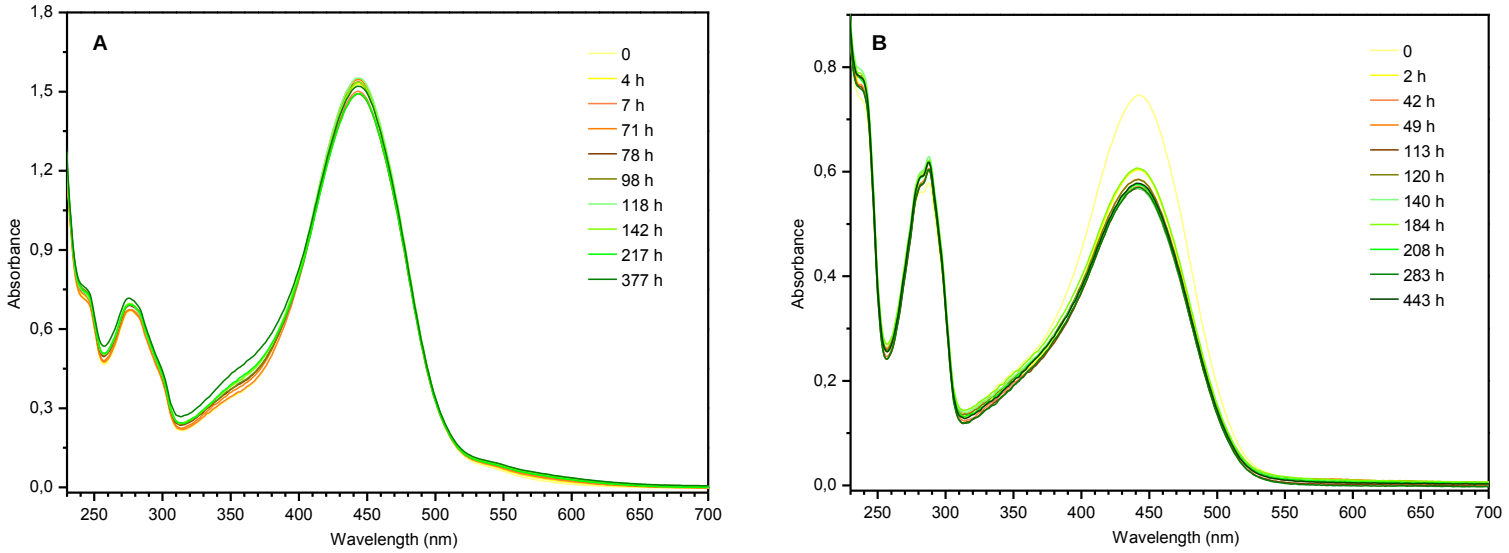


**Figure A3.** Absorption spectra of brazilein $10^{-4}$ M in **A)** MeOH and **B)** $H_2O$:MeOH (70:30, v/v) at pH 2.9.

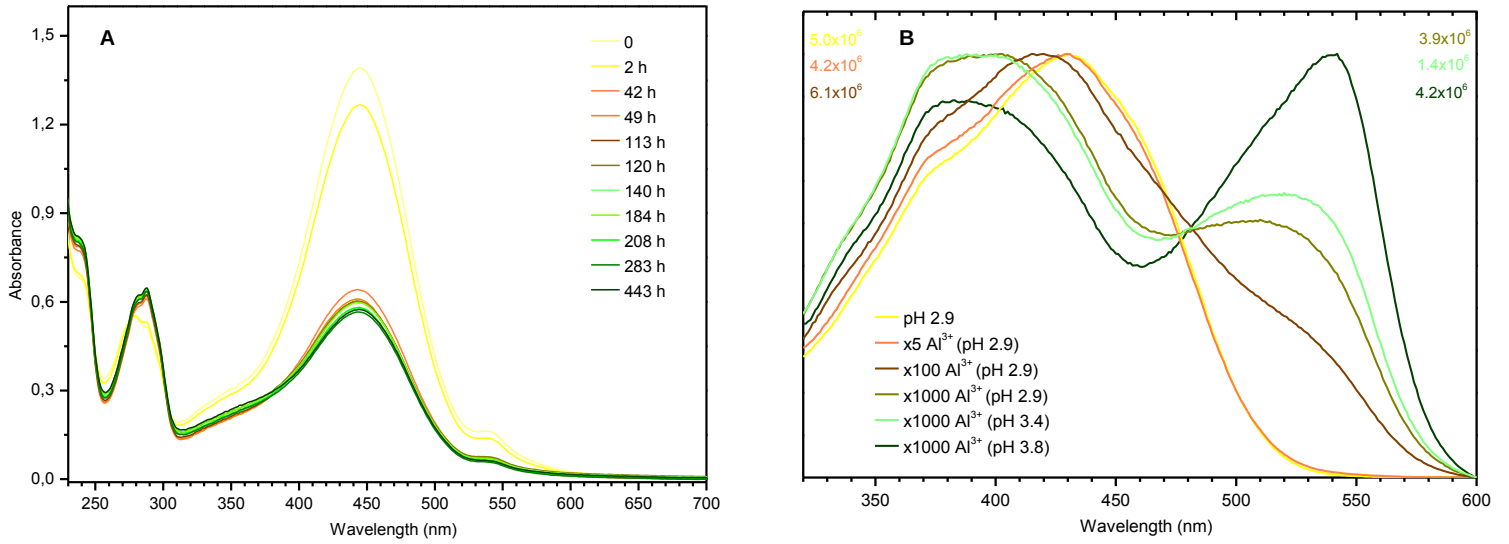


**Figure A4. A)** Absorption spectra of brazilein $10^{-4}$ M in $H_2O$:MeOH (70:30, v/v) at pH 6.1 and **B)** effect of the $Al^{3+}$ ratio and pH on the fluorescence spectra of brazilein $10^{-5}$ M in $H_2O$:MeOH (70:30, v/v).

**APPENDIX 4.** HPLC-DAD analysis

### 4.1. HPLC-DAD analysis of four different species

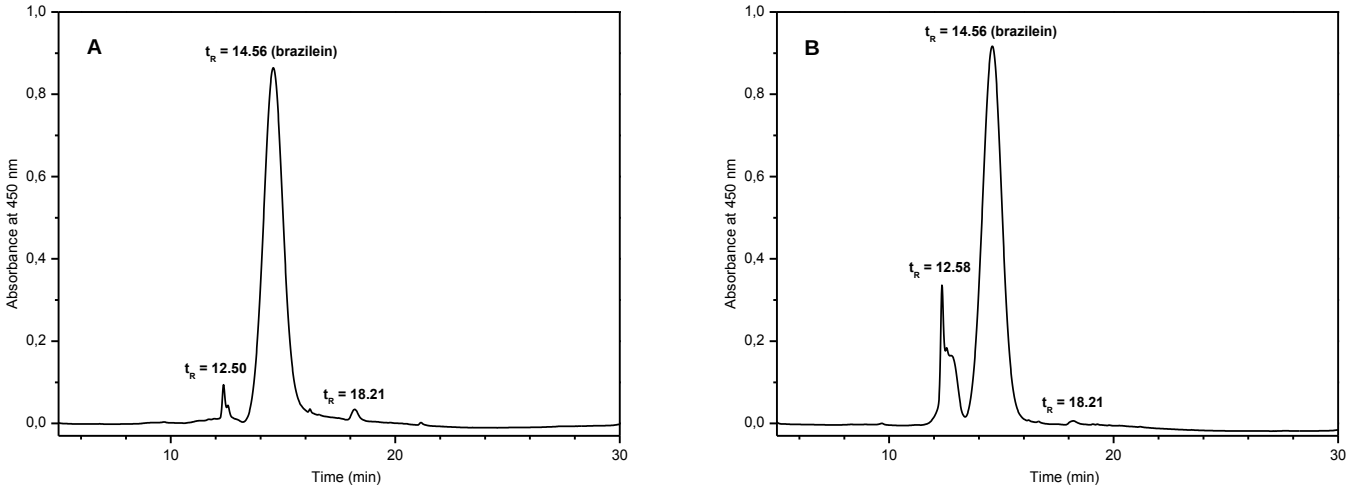


**Figure A5.** HPLC-DAD chromatogram of **A)** *Caesalpinia echinata* and **B)** *Caesalpinia sappan* at 450 nm.

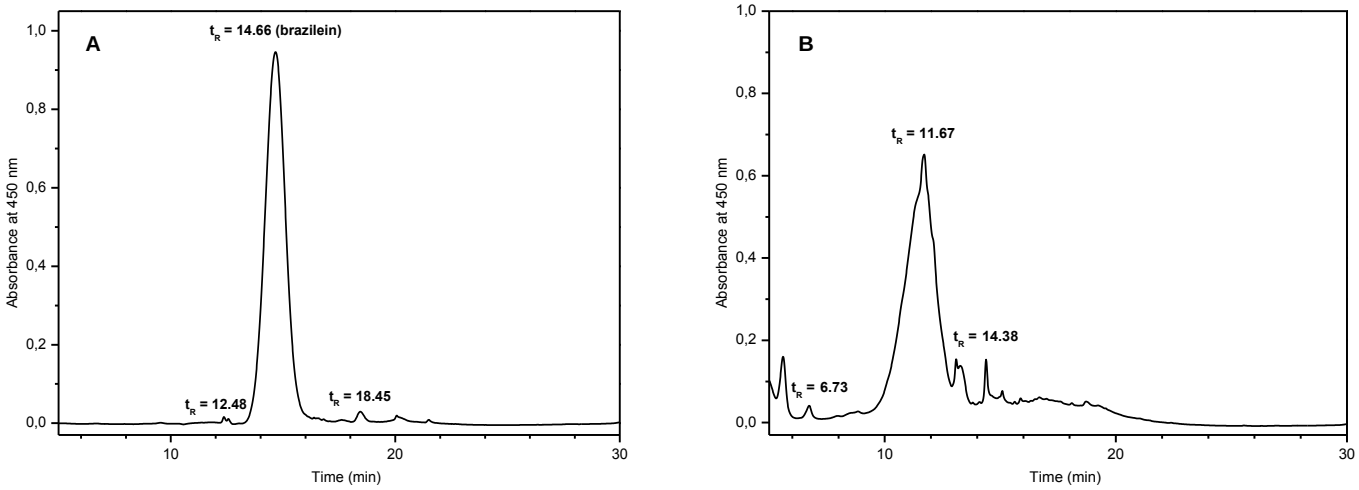


**Figure A6.** HPLC-DAD chromatogram of **A)** *Haematoxylum brasiletto* and **B)** *Haematoxylum campechinium* at 450 nm.

The HPLC-DAD chromatograms of *C. echinata*, *C. sappan* and *H. brasiletto* at 450 nm (Figures A5 and A6.A), revealed three main retention peaks, which vary in their relative quantity: a large retention peak at 14.56 min (14.66 min for the latter species), a poorly resolved peak at around 12.50 min and a very small one at *ca.* 18.30 min. The larger peak at 14.56 min is more intense for *H. brasiletto* and smaller for *C. echinata*, while the poorly resolved peak at around 12.50 min is more intense for *C. sappan* and very small for *H. brasiletto*. With respect to UV-vis spectra (Figures A7 and A8), six different types can be distinguished. At 10.6 min an UV-Vis spectrum, corresponding to a very small retention peak and with a main band at 285 nm is observed. According to the study carried out by Nowik (2001) [18], the latter may correspond to the “type D” spectra. At 12.5 min, a spectrum similar to that of brazilein, with $\lambda_{max}$ at 283 and 400 nm is obtained. On the left side of the large peak at 14.56 min, an UV-Vis spectrum with three absorptions bands at 218, 252 and 284 nm is observed. According to Nowik (2001) [18], the latter corresponds to the “type A” spectra. At 14.60 min an UV-Vis spectrum with $\lambda_{max}$ at 446 nm, identical to that attributed to the red chromophore brazilein is obtained. On the right side of this peak and at 18.2 min UV-Vis spectra of unidentified species are observed.

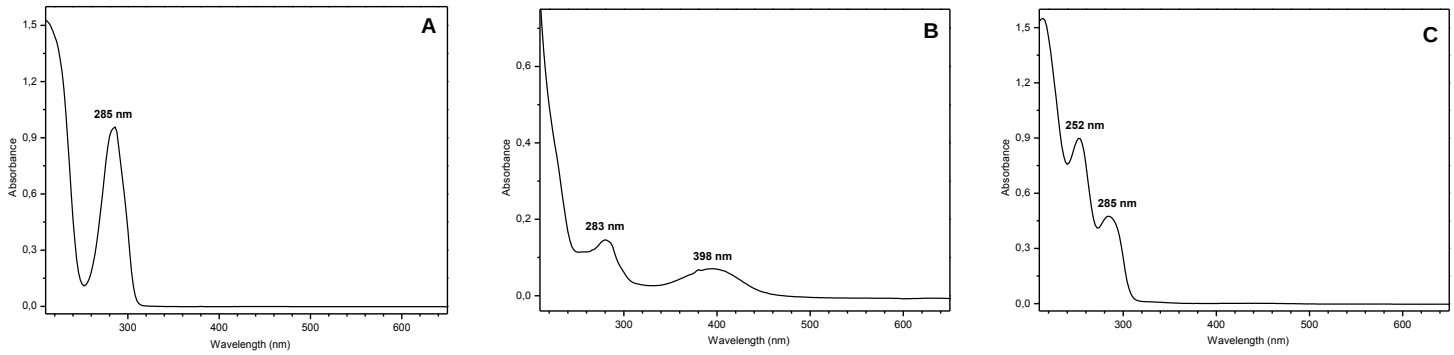


**Figure A7.** UV-Vis spectra at **A)** 10.56 ("type D" spectrum), **B)** 12.50 and **C)** 13.00 ("type A" spectrum) min.

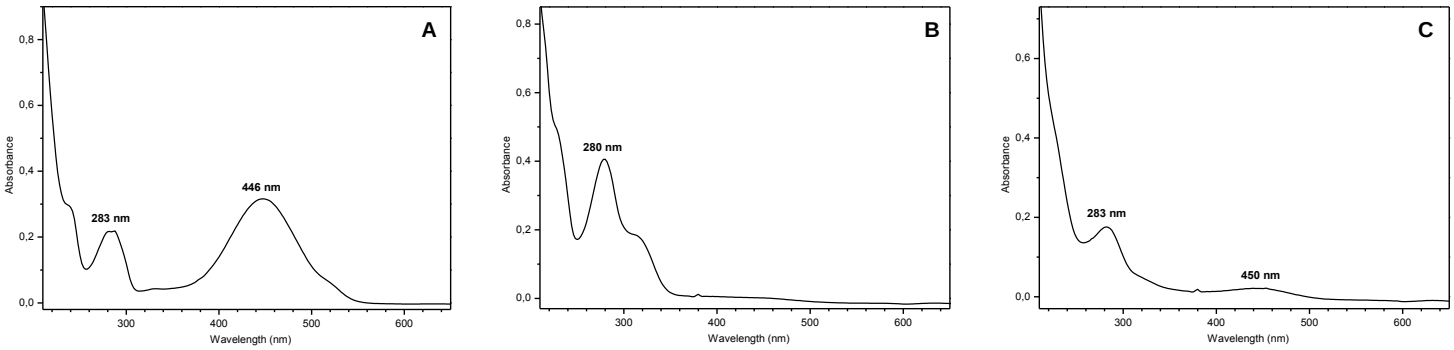


**Figure A8.** UV-Vis spectra at **A)** 14.60 (brazilein's spectrum), **B)** 16.70 and **C)** 18.21 min.

The HPLC-DAD chromatogram of *H. campechinium* at 450 nm (Figure A6.B) differs from those of the other three species. In this case, retention peaks at 5.56, 6.73, 11.67, 13.35 and 14.38 min are found. Although the UV-Vis spectra related to these peaks are somewhat similar to those presented in Figures A7 and A8, *H. campechenium* can be clearly differentiated from *C. echinata*, *C. sappan* and *H. brasiletto* since different retention times are observed.

### 4.2. HPLC-DAD analysis of two medieval brazilwood lake pigments reconstructions

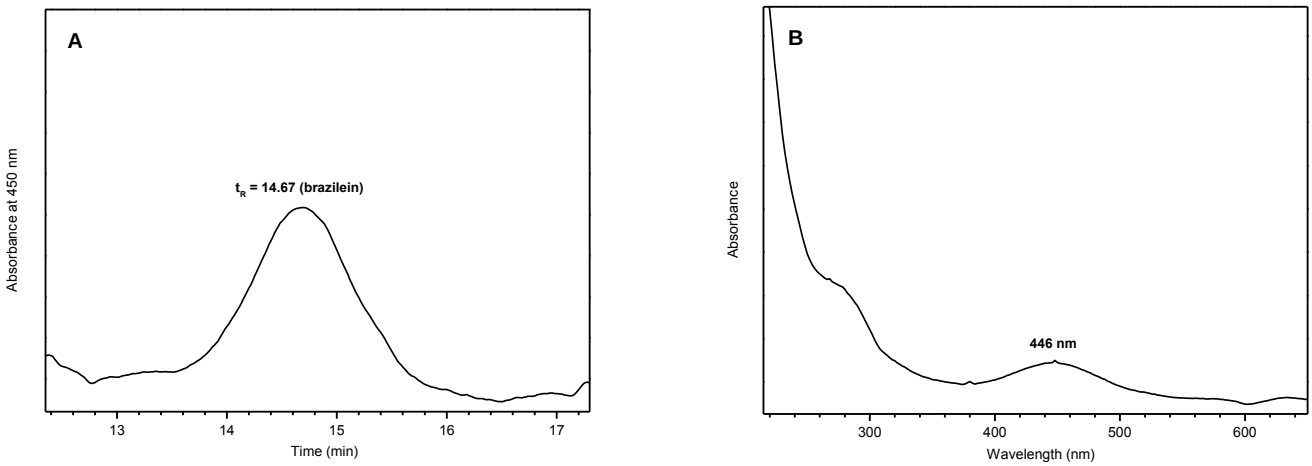


**Figure A9. A)** HPLC-DAD chromatogram of chapter 8, exp. 6 at 450 nm and **B)** UV-Vis spectrum at 14.60 min.

The HPLC-DAD chromatograms of the pigments extraction (chapter 8, exp. 6 and chapter 9, exp.5) at 450 nm (Figure A9) revealed only a main large retention peak at 14.56 min, which corresponds to that of brazilein. This indicates that the main red chromophore present in the pigments is, in fact, brazilein.

**APPENDIX 5.** Measures conversion and scaling down of materials quantities to laboratory scale

The extrapolation of the ingredient quantities from the documentary sources was performed considering the following conversion table:

**Table A1.** Conversion table[iii].

| Measures | Abbreviations | S.I. Units |
|---|---|---|
| 1 gallon | 1 gal. | 4540 mL |
| 1 ounce | 1 oz. | 28.35 g |
| 1 pound | 1 lb. | 453.6 g |
| 1 hundredweight | 1 cwt. | 50800 g |
| 1 pennyweight | | 1.555 g |

Extrapolation was carried out always considering the same brazilwood starting quantity (5.0 g). For each case, brazilwood in the form of scrapes as it is sold today was ground in a coffee grinder and used as such[iv].

The conversion of ingredients quantities from the four *Livro de como se fazem as cores* recipes to S.I. units as well as the materials used are presented in Tables A2 – A5.

**Table A2.** Description of chapter 8 recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| Brazilwood | *Caesalpinia echinata* | | 1 once | 28.35 g | 5.0 g |
| Powdered alum | Aluminium potassium sulphate dodecahydrate | $AlK(SO_4)_2{\cdot}12H_2O$ | ¼ once | 7.10 g | 1.25 g |
| Powdered lead white | Basic lead carbonate | $2PbCO_3{\cdot}Pb(OH)_2$ | 2 pennyweights | 3.11 g | 0.55 g |
| Urine | Urine (pH 7) | | "until they are covered" | | 100 mL |
| Chalk-stone | Calcium carbonate | $CaCO_3$ | | | |
| Gypsum | Calcium sulphate dihydrate | $CaSO_4{\cdot}2H_2O$ | | | |

**Table A3.** Description of chapter 9 recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| Brazilwood | *Caesalpinia echinata* | | "as much as you need" | | 5.0 g |
| Lye of vine branches | Wood ashes solution (pH 11-12) | | "so that the brazil wood is covered with it" | | 100 mL |
| Powdered alum | Aluminium potassium sulphate dodecahydrate | $AlK(SO_4)_2{\cdot}12H_2O$ | "two parts" | | *See table A11* |
| Powdered chalk | Calcium carbonate | $CaCO_3$ | "at least a half a part" | | *See table A11* |

[iii] Carlyle, L. 2001. *The Artist's Assistant*. London: Archetype Publications.

[iv] Grinding brazilwood in a mortar was tried but without any success due to the hardness of the wood.

**Table A4.** Description of chapter 27 recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| Brazilwood | *Caesalpinia echinata* | | "as much as you want" | | 5.0 g |
| Alum | Aluminium potassium sulphate dodecahydrate | $AlK(SO_4)_2{\cdot}12H_2O$ | | | 1.5 g |
| Urine of a chaste man | Urine (pH 7) | | "until they are well covered" | | 100 mL |
| A piece of chalk | Calcium carbonate | $CaCO_3$ | "throw some of its powder (…) until it seems that there is as much of one as of the other" | | *See table A12* |

**Table A5.** Description of chapter 44 recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| Brazilwood | *Caesalpinia echinata* | | | | 5.0 g |
| Virgin lime clear water | Calcium oxide solution (pH 11-12) | | | | 100 mL |
| Alum | Aluminium potassium sulphate dodecahydrate | $AlK(SO_4)_2{\cdot}12H_2O$ | "a little" | | 0.5 g |

The conversion of ingredients quantities from the four W&N 19th century recipes to S.I. units as well as the materials used are presented in Tables A6 – A9. All materials and quantities were confirmed in the W&N database, which it incorporates translations of terms and materials to modern equivalents as well as automatic conversion of British measures to S.I. units.

**Table A6.** Description of Rose Pink recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| Brazilletta | *Caesalpinia echinata* | | 2½ lbs | 1134 g | 1.0 g |
| West India Peach Wood | *Caesalpinia echinata* | | ½ lbs | 226.8 g | |
| Boiling water | Boiling water (pH 6) | | | | 1500 mL |
| Cream of tartar | Potassium hydrogen tartrate | $C_4H_5KO_6$ | 1 lb | 453.6 g | 0.33 g |
| Powdered alum | Aluminium potassium sulphate dodecahydrate | $AlK(SO_4)_2{\cdot}12H_2O$ | 1 cwt | 50800 g | 37.33 g |
| Whiting | Calcium carbonate | $CaCO_3$ | 2½ cwt | 127000 g | 93.33 g |

**Table A7.** Description of Rose Pink II recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| J(S)apan wood | *Caesalpinia echinata* *Caesalpinia sappan* | | 300 lbs | 136080 g | 5.0 g |
| Alum | Aluminium potassium sulphate dodecahydrate | $AlK(SO_4)_2{\cdot}12H_2O$ | 148 lbs | 67132.8 g | 2.47 g |
| Whiting | Calcium carbonate | $CaCO_3$ | 264 lbs | 119750.4 g | 4.4 g |

**Table A8.** Description of Rose Pink Seacy's Formula recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| (Nicaragua) wood | *Caesalpinia echinata* *Haematoxylum brasiletto* | | 10½ lbs | 4762.8 g | 5.0 g |
| Boiling water | Boiling water (pH 6) | | 16 galls | 72640 mL | 76 mL |
| Powdered alum dissolved in hot water | Aluminium potassium sulphate dodecahydrate dissolved in hot water | $AlK(SO_4)_2{\cdot}12H_2O$ | 10 lbs | 4536 g | 4.76 g in 50 mL |
| Solution of permuriate of tin | Tin(IV) chloride pentahydrated dissolved in water | $SnCl_4{\cdot}5H_2O$ | 10 lbs | 4536 g | 4.76 g in 50 mL |
| Pearlash dissolved in hot water | Potassium carbonate dissolved in hot water | $K_2CO_3$ | ¼ lb in 2 galls | 113.4 g in 9080 mL | 0.12 g in 10 mL |
| Alum dissolved in boiling water | Aluminium potassium sulphate dodecahydrate dissolved in boiling water | $AlK(SO_4)_2{\cdot}12H_2O$ | 30 lbs in 20 galls | 13608 g in 90800 mL | 14.29 g in 95 mL |
| Whiting in boiling water | Calcium carbonate dissolved in boiling water | $CaCO_3$ | 40 lbs | 18144 g | 19.05 g in 10 mL |

**Table A9.** Description of Wood Purple recipe materials and quantities.

| Material original name | Material used | Chemical formula | Original quantity | S.I. Units | Quantity used |
|---|---|---|---|---|---|
| Lima peachwood | *Caesalpinia echinata* | | 3½ lbs | 1587.6 g | 5.0 g |
| Boiling water | Boiling water (pH 6) | | 6 galls | 27240 mL | 86 mL |
| Cold water | Water (pH 6) | | 15 galls | 68100 mL | 215 mL |
| Carbonate of soda | Sodium carbonate | $Na_2CO_3$ | 6 lbs | 2721.6 g | 8.57 g |
| Powdered alum | Aluminium potassium sulphate dodecahydrate | $AlK(SO_4)_2{\cdot}12H_2O$ | 6 lbs | 2721.6 g | 17.0 g |

**APPENDIX 6.** Reconstructions of brazilwood lake pigments

### 6.1. Medieval brazilwood lake pigments reconstructions

All information concerning the preparation of medieval brazilwood lake pigments are presented in Tables A10 – A13[v].

**Table A10.** Information on the reconstructions of chapter 8 from *Livro de como se fazem as cores* (starting with 5.0 g of brazilwood and 100 mL of urine, stirring 5/6 times for three days; alum and lead white were ground together)

| exp. | Alum (g) | Lead white (g) | pH of extraction | Filtration | Chalk | Gypsum | Quantity (g) | η (%) [¥] | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.25 | 0.55 | 4.3 | | | • | 0.42 | - | 38 32 15 | 53 25 9 |
| 2 | 1.25 | 0.55 | 4.3 | • | | • | 0.53 | - | 39 36 11 | 35 37 12 |
| 3 | 1.25 | 0.55 | 4.5 | • | • | | 0.42 | - | 32 35 -5 | 27 31 -5 |
| 4 | 0.90 | 0.55 | 5.5 | • | | • | 1.39 | - | 41 31 3 | 52 28 -3 |
| 5 | 1.25 | 1.00 | 5.3 | • | | • | 0.50 | - | 53 33 3 | 50 30 -8 |
| 6 | 1.25 | 0.55 | 4.2 | • | | • | 0.82 | - | 61 29 9 | 55 31 -6 |
| 7 | 2.00 | 0.55 | 3.8 | • | | • | 1.16 | - | 42 35 -2 | 41 34 -13 |

[¥] Yield was not calculated since chalk and gypsum quantities were not weighted.

**Table A11.** Information on the reconstructions of chapter 9 from *Livro de como se fazem as cores* (starting with 5.0 g of brazilwood and 100 mL of lye left to boil for three hours; alum and chalk were ground first individually and then together).

| exp. | pH of extraction | Filtration | Alum (g) | Chalk (g) | Gypsum | Final pH | Quantity (g) | η (%) [¥] | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.0 | • | 0.5 | 0.5 | | 6.4 | 0.30 | 30.0 | | |
| 2 | 9.0 | • | 1.0 | 0.25 | | 6.2 | 0.50 | 40.0 | 37 25 2 | 31 20 -2 |
| 3 | 10 | • | 1.0 | 0.75 | | 7.0 | 0.80 | 45.7 | | |
| 4 | 10 | | 1.25 | 0.75 | | 7.0 | 0.50 | 7.10 | 33 22 -3 | 31 22 -5 |
| 5 | 10 | • | 1.25 | 1.0 | • | | 2.15 | - | 50 24 1 | 41 24 -5 |
| 6 | 6.0 | • | 1.0 | 0.75 | | 7.0 | 1.34 | 76.6 | 26 18 6 | 30 20 5 |
| 7 | 6.0 | • | 1.0 | 0.25 | | 6.9 | 0.86 | 68.8 | 26 20 8 | 28 19 6 |

[¥] Yield was calculated only using alum and chalk quantities, except for experience 4 in which no filtration was performed (in this case, brazilwood quantity was also considered to calculate the yield).

[v] Final product quantities were measured after washing and drying of pigment.

"L*a*b* (Ga)" refers to colour coordinates of pigments painted with gum-arabic and "L*a*b* (Ew)" refers to those painted with egg white. In both cases, paints were applied on parchment.

**Table A12.** Information on the reconstructions of chapter 27 from *Livro de como se fazem as cores* (starting with 5.0 g of brazilwood and 100 mL of urine, standing for three days).

| exp. | Alum (g) | pH of extraction | Filtration | Chalk (g) | Final pH | Quantity (g) | η (%) ¥ | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.5 | 4.0 | | 20 | 6.1 | 15.2 | 57.4 | 62 17 -13 | 63 18 -12 |
| 2 | 1.5 | 4.0 | • | 10 | 6.0 | 4.50 | 39.1 | 61 29 -7 | 63 27 -11 |
| 3 | 1.5 | 4.3 | • | 1.5 | 5.8 | 0.30 | 10.0 | 63 28 -7 | 62 27 -11 |
| 4 | 1.5 | 4.0 | • | 2.0 | 6.5 | 2.20 | 62.9 | 45 51 5 | 48 47 -1 |

¥ Yield was calculated only using alum and chalk quantities, except for experience 1 in which no filtration was performed (in this case, brazilwood quantity was also considered to calculate the yield).

**Table A13.** Information on the reconstructions of chapter 44 from *Livro de como se fazem as cores* (starting with 5.0 g of brazilwood and 100 mL of lime).

| exp. | pH of extraction | Filtration | Alum (g) | Final pH | Quantity (g) | η (%) ¥ | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|
| 1 | 9.5 | | 0.5 | 7.0 | 2.18 | 39.6 | | |
| 2 | 9.5 | • | 0.5 | 7.3 | 0.10 | 20.0 | 55 23 5 | 35 27 1 |
| 3 | 10.5 | • | 0.5 | 6.2 | 0.12 | 24.0 | 57 24 10 | 35 26 -3 |
| 4 | 10 | • | 0.5 | 6.1 | 0.30 | 60.0 | 39 42 17 | 36 44 15 |

¥ Yield was calculated only using alum quantity, except for experience 1 in which no filtration was performed (in this case, brazilwood quantity was also considered to calculate the yield).

### 6.2. Nineteenth-century brazilwood lake pigments reconstructions

All information concerning the preparation of 19th century brazilwood lake pigments are presented in Tables A14 – A17[vi].

**Table A14.** Information on the reconstruction of Rose Pink recipe from the W&N 19th century archive (starting with 1.0 g of brazilwood, boiling for one hour).

| exp. | Boiling water (mL) | pH of extraction | Filtration | Cream of tartar (g) | pH | Alum (g) | pH | Chalk (g) | Final pH | Quantity (g) | η (%) ¥ | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rose Pink | 1500 | 4.2 | • | 0.33 | 3.4 | 37.33 | 2.5 | 93.33 | 5.9 | 84.5 | 64.5 | 81 23 3 | 78 28 3 |

¥ Yield was calculated only using cream of tartar, alum and chalk quantities.

[vi] Final product quantities were measured after washing and drying of pigment.
"L*a*b* (Ga)" refers to colour coordinates of pigments painted with gum-arabic and "L*a*b* (Ew)" refers to those painted with egg white. In both cases, paints were applied on parchment.

**Table A15.** Information on the reconstructions of Rose Pink II recipe from the W&N 19th century archive (starting with 5.0 g of brazilwood, boiling for one hour).

| exp. | Boiling water (mL) | pH of extraction | Filtration | Alum (g) | pH | Chalk (g) | Final pH | Quantity (g) | η (%) ¥ | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rose Pink II | 80 | 4.5 | • | 2.47 | 3.0 | 4.4 | 6.8 | 5.23 | 76.1 | 39 46 12 | 46 46 2 |
| Rose Pink II (b) | 80 | 5.0 | • | 2.47 | 3.0 | 4.4 | 6.7 | 5.18 | 75.4 | 42 45 10 | 39 40 7 |

¥ Yield was calculated only using alum and chalk quantities.

**Table A16.** Information on the reconstructions of Rose Pink. Seacy's Formula recipe from the W&N 19th century archive (starting with 5.0 g of brazilwood, boiling and stirring for one hour, and repeating twice more).

| exp. | Boiling water (mL) | pH of extraction | Filtra-tion | Alum (g) | pH | Permuriate of tin(IV) (g) | pH | Potassium carbonate (g) | pH | Chalk (g) | Alum (g) | Quantity (g) | η (%) ¥ | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rose Pink Seacy's | 76 | 4.5 | • | 4.76 | 2.6 | 4.76 | 0.6 | 0.12 | 0.7 | 19.05 | 14.29 | 26.0 | 60.5 | 68 39 10 | 76 27 12 |
| Rose Pink Seacy's (b) | 76 | 4.4 | • | 4.76 | 2.5 | 4.76 | 0.6 | 0.12 | 0.7 | 19.05 | 14.29 | 26.9 | 62.6 | 61 44 10 | 65 39 8 |

¥ Yield was calculated only using alum, permuriate of tin(IV), potassium carbonate and chalk quantities.

**Table A17.** Information on the reconstruction of Wood Purple recipe from the W&N 19th century archive (starting with 5.0 g of brazilwood, boiling for one hour).

| exp. | Boiling water (mL) | Cold water (mL) | pH of extraction | Filtration | Carbonate of soda (g) | pH | Alum (g) | Final pH | Quantity (g) | η (%) ¥ | L*a*b* (Ga) | L*a*b* (Ew) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wood Purple | 86 | 215 | 4.7 | • | 8.57 | 10.9 | 17.0 | 7.0 | 7.25 | 28.4 | 38 47 9 | 45 45 1 |

¥ Yield was calculated only using carbonate of soda and alum quantities.

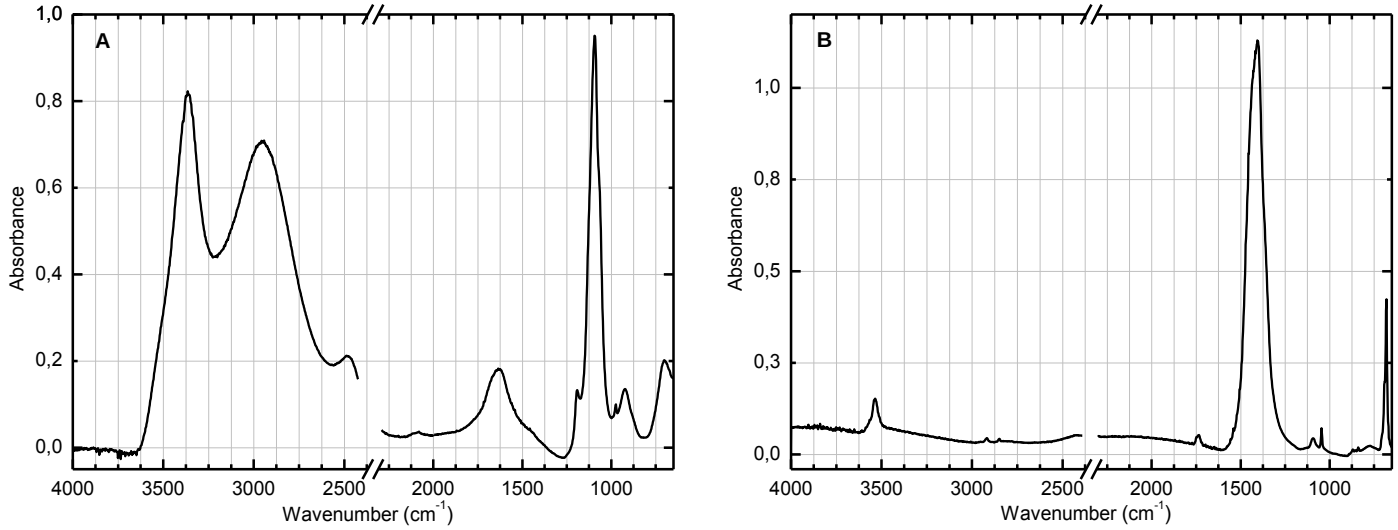


**Figure A10.** Infrared spectra of **A)** aluminium potassium sulphate dodecahydrate and **B)** Flake white lead.

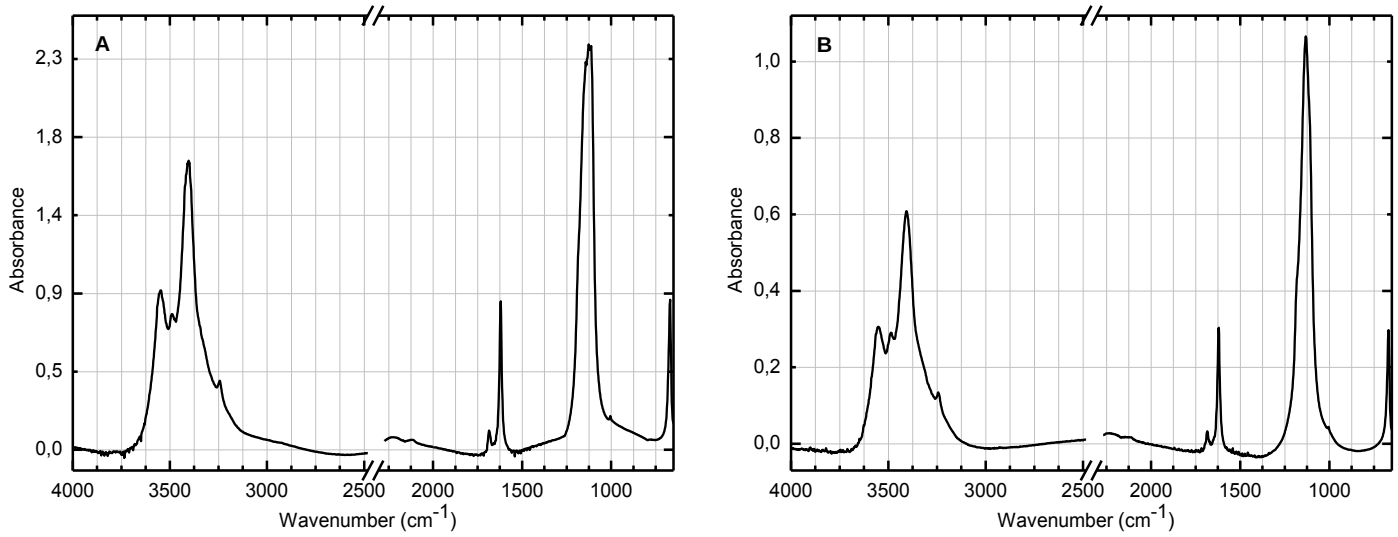


**Figure A11.** Infrared spectra of **A)** calcium sulphate dihydrate and **B)** calcium sulphate dihydrate from bowl used during some pigments' manufacture.

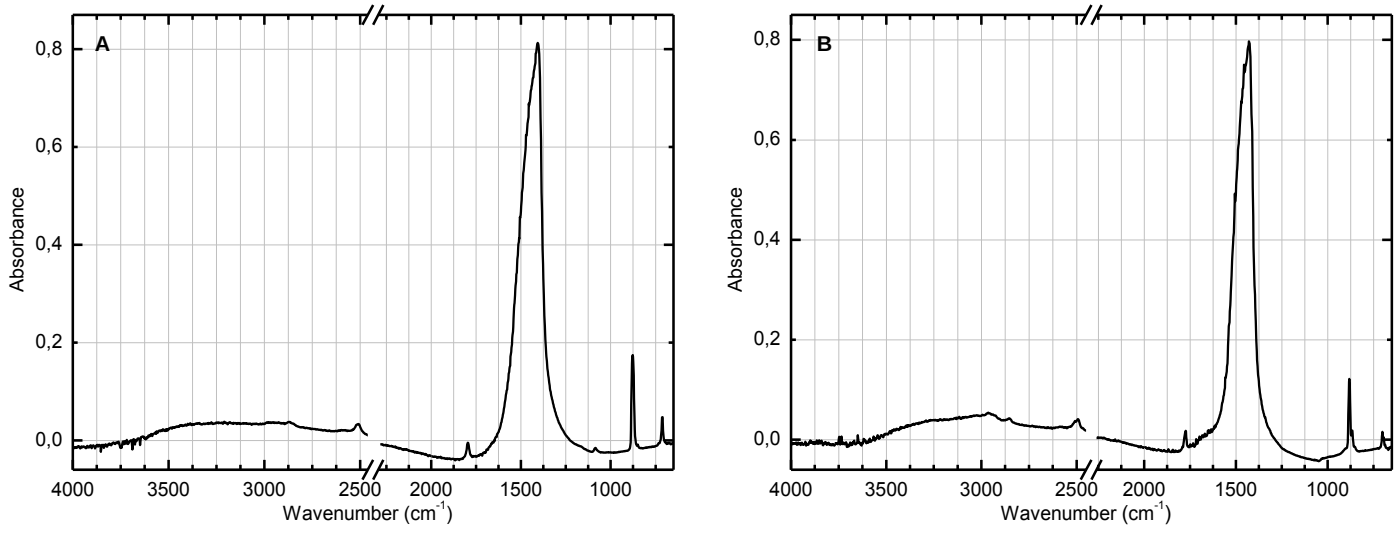


**Figure A12.** Infrared spectra of **A)** calcium carbonate and **B)** sodium carbonate.

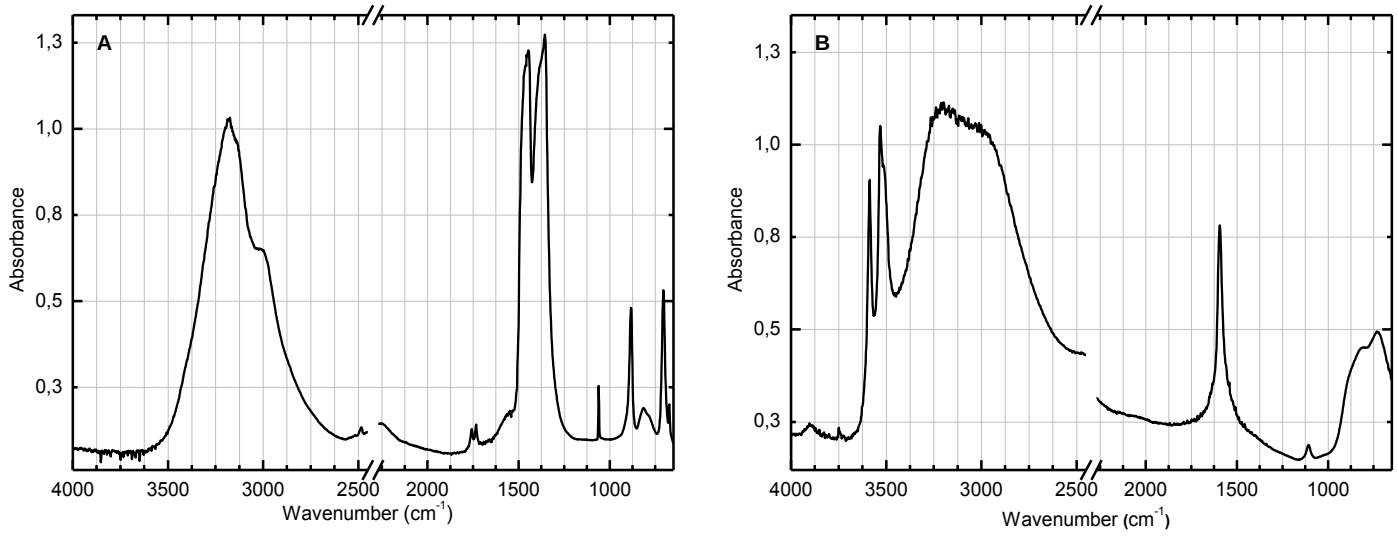


**Figure A13.** Infrared spectra of **A)** potassium carbonate and **B)** tin(IV) chloride pentahydrate.

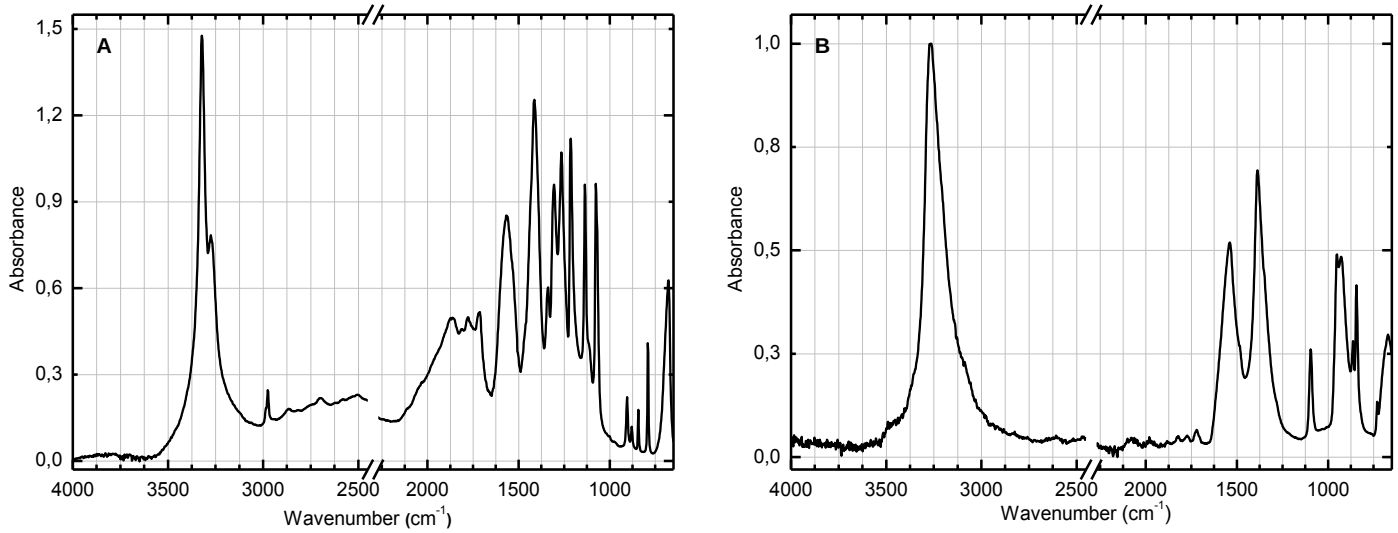


**Figure A14.** Infrared spectra of **A)** potassium hydrogen tartrate and **B)** sodium aluminium hydroxyl carbonate (dawsonite)[vii].

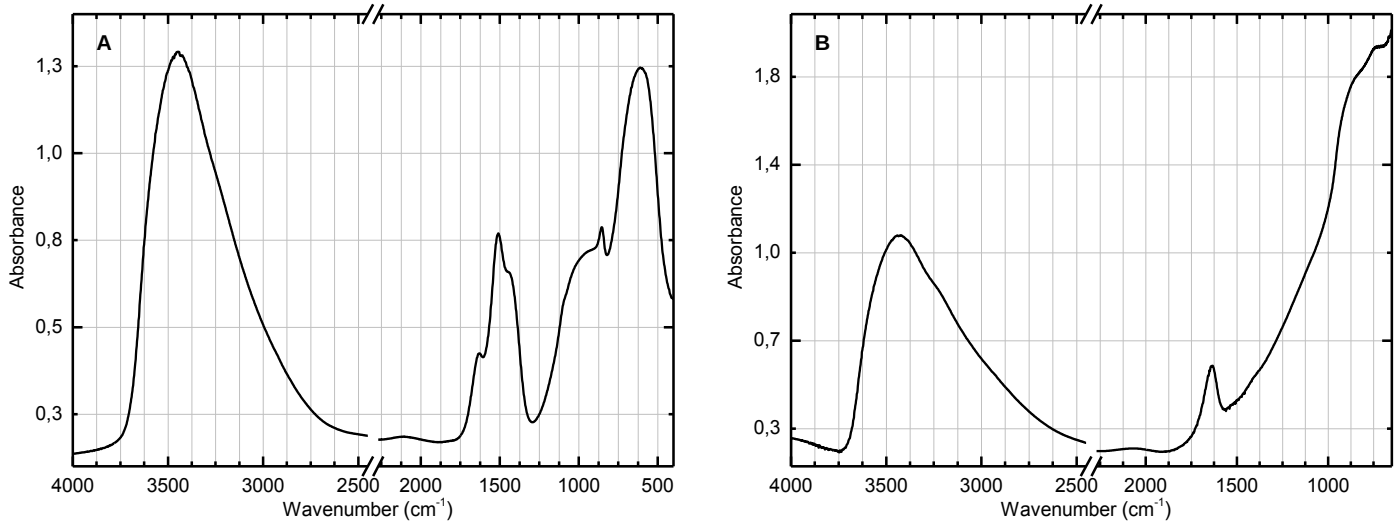


**Figure A15.** Infrared spectra of **A)** aluminium hydroxide and **B)** aluminium oxide.

[vii] Dawsonite R050641 from RRUFF Database [accessed in September 2012]. Available at: <http://rruff.info/dawsonite/display=default/R050641>

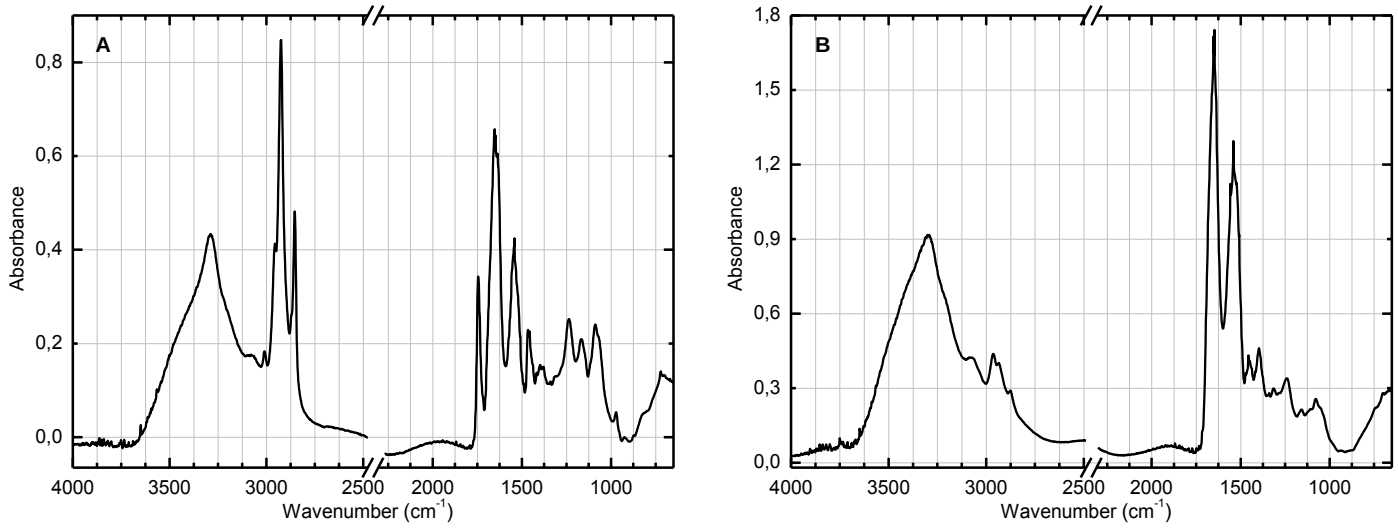


**Figure A16.** Infrared spectra of **A)** egg yolk and **B)** egg white.

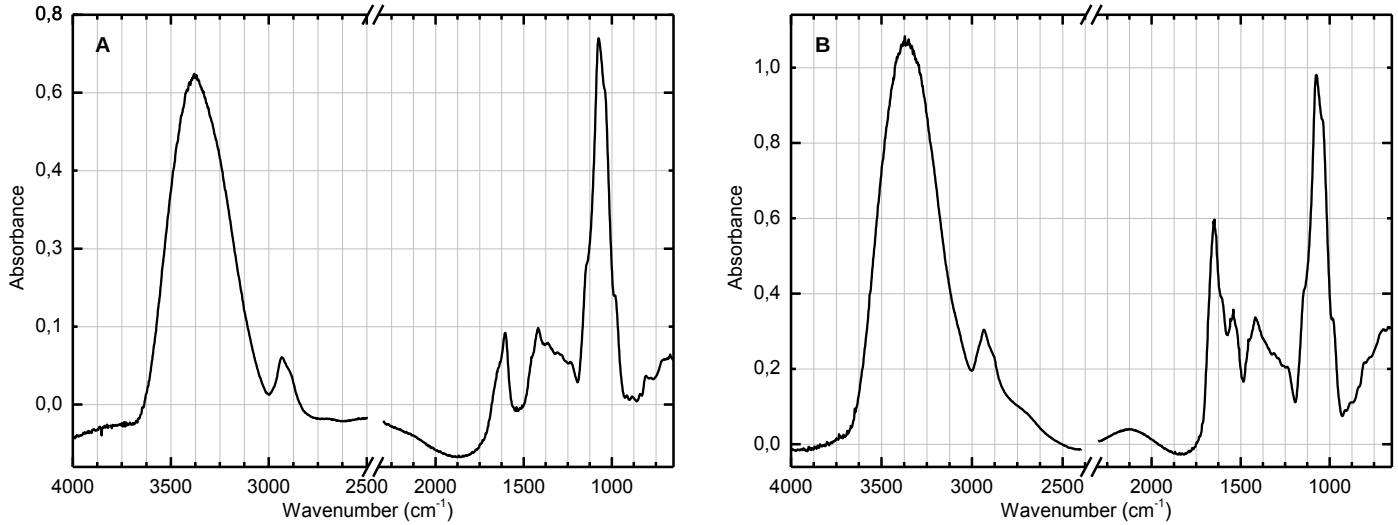


**Figure A17.** Infrared spectra of **A)** gum-arabic and **B)** gum-arabic:egg white (3:1).

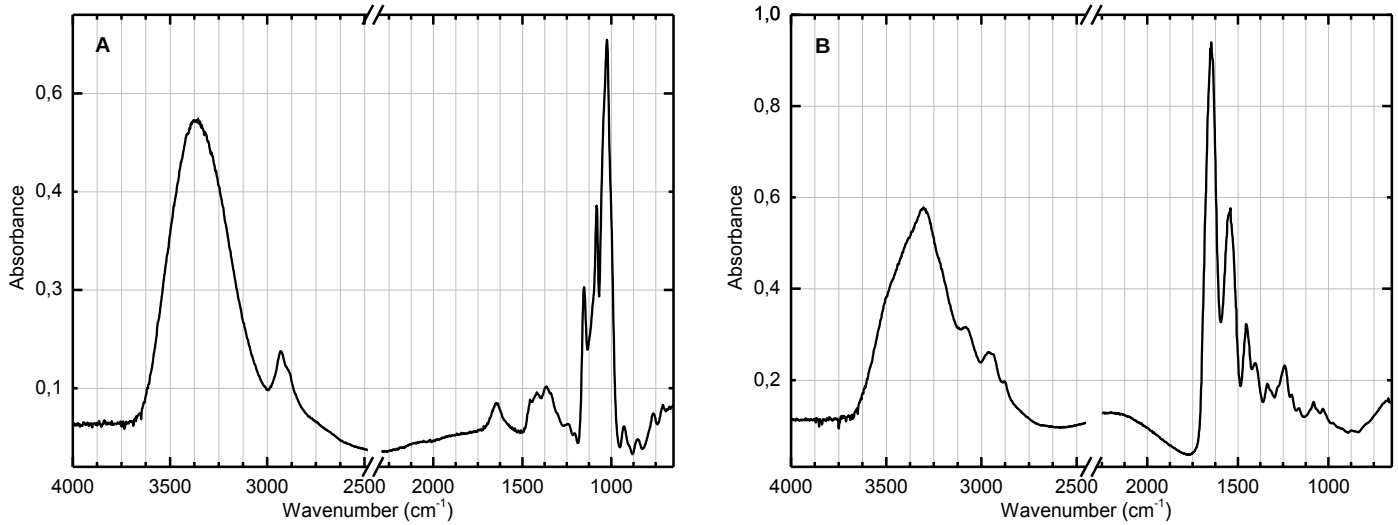


**Figure A18.** Infrared spectra of **A)** transparent starch solution and **B)** parchment glue.

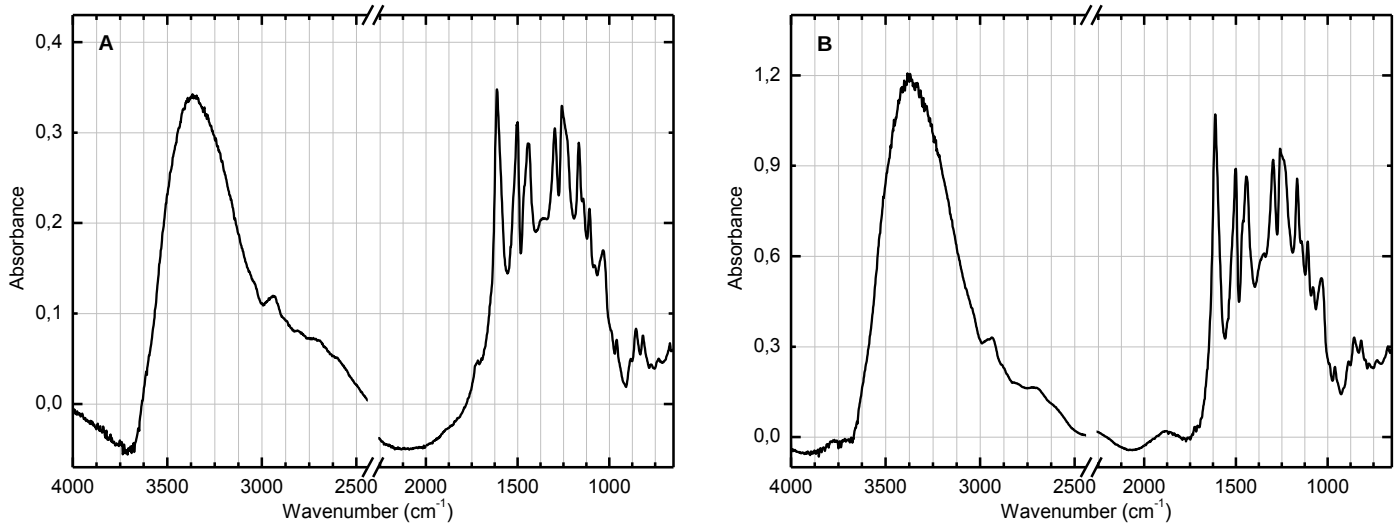


**Figure A19.** Infrared spectra of aqueous extract from **A)** *Caesalpinia sappan* and **B)** *Caesalpinia echinata*.

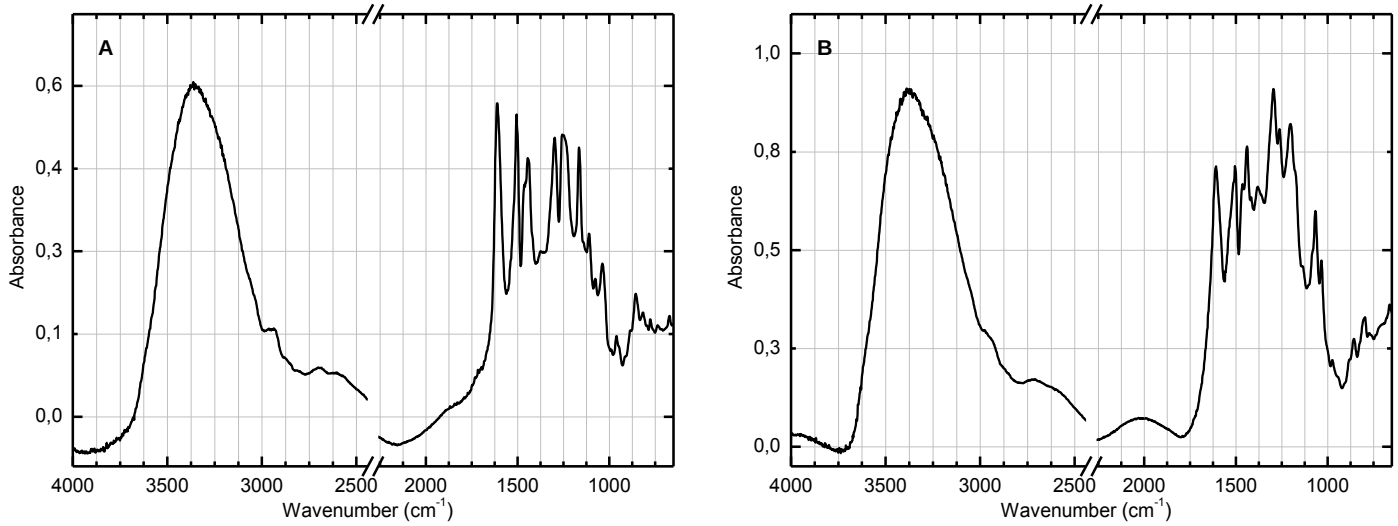


**Figure A20.** Infrared spectra of aqueous extract from **A)** *Haematoxylum brasiletto* and **B)** *Haematoxylum campechianum*.

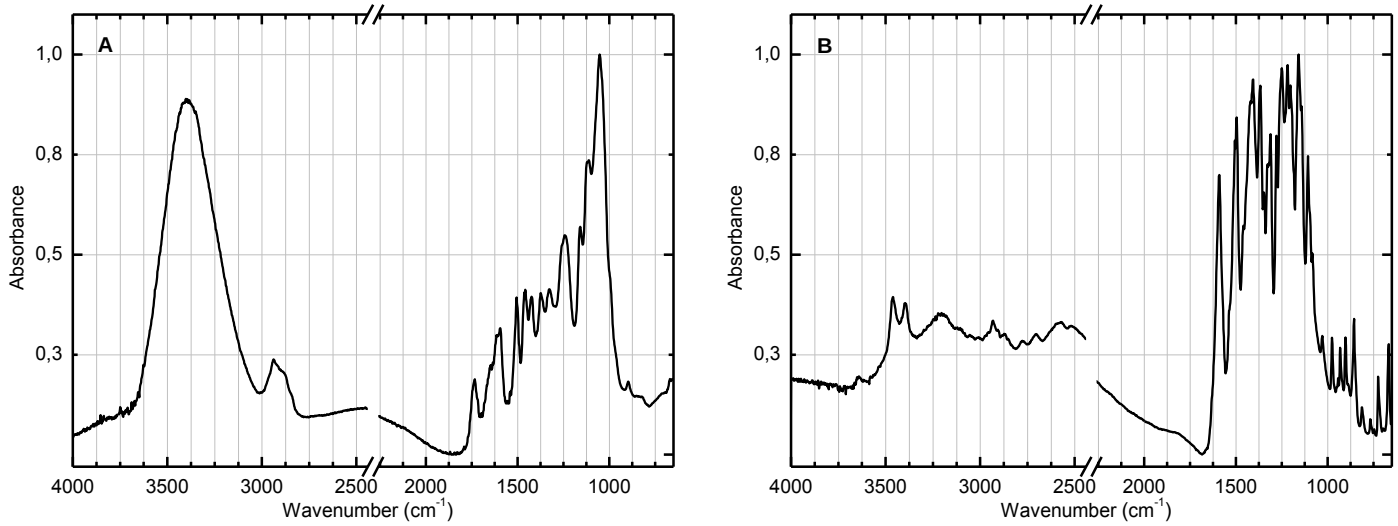


**Figure A21.** Infrared spectra of **A)** *Caesalpinia echinata* wood and **B)** brazilein.

**APPENDIX 8.** Infrared spectra of brazilwood lake pigments reconstructions

## 8.1. Infrared spectra of medieval brazilwood lake pigments reconstructions

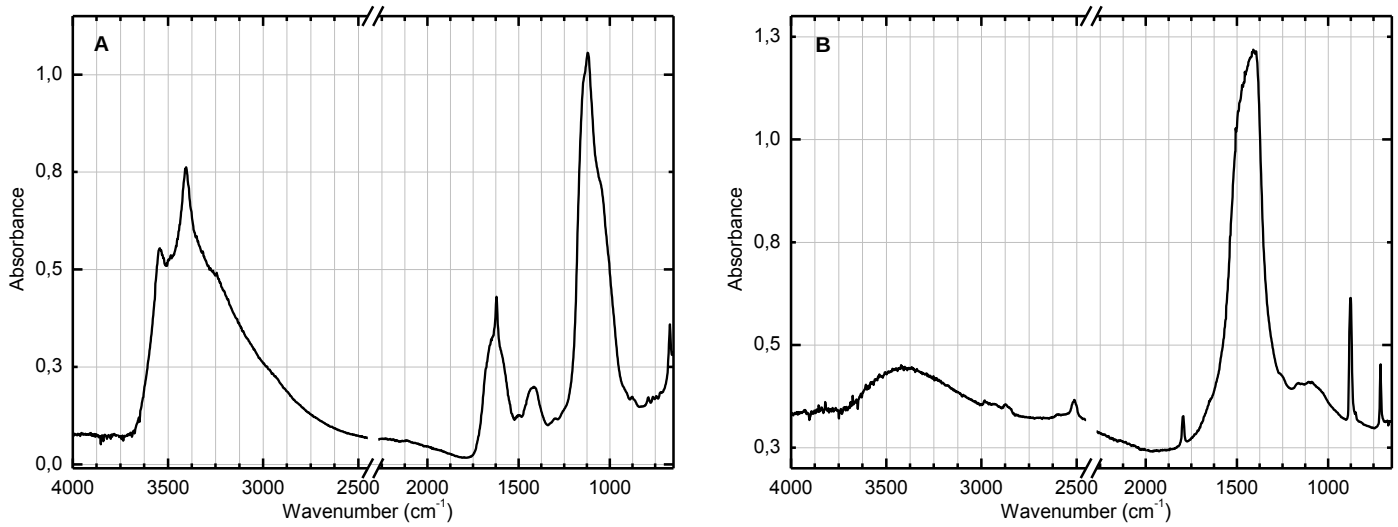


**Figure A22.** Infrared spectra of **A)** chapter 8, exp. 2 and **B)** chapter 8, exp. 3 pigment.

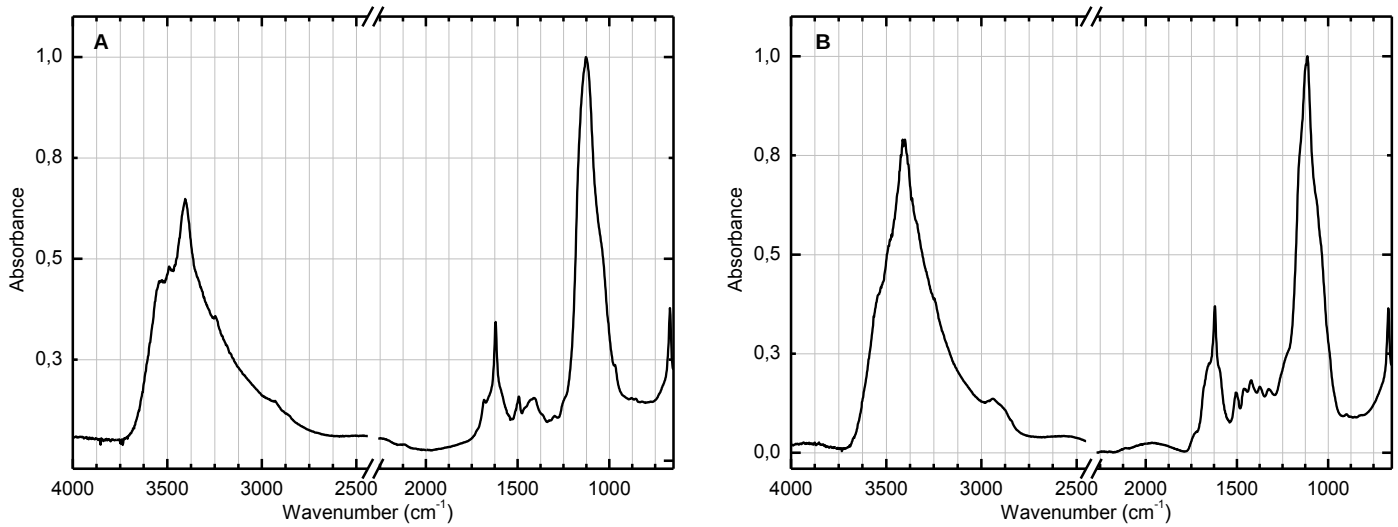


**Figure A23.** Infrared spectra of **A)** chapter 8, exp. 5 and **B)** chapter 8, exp. 6 pigment.

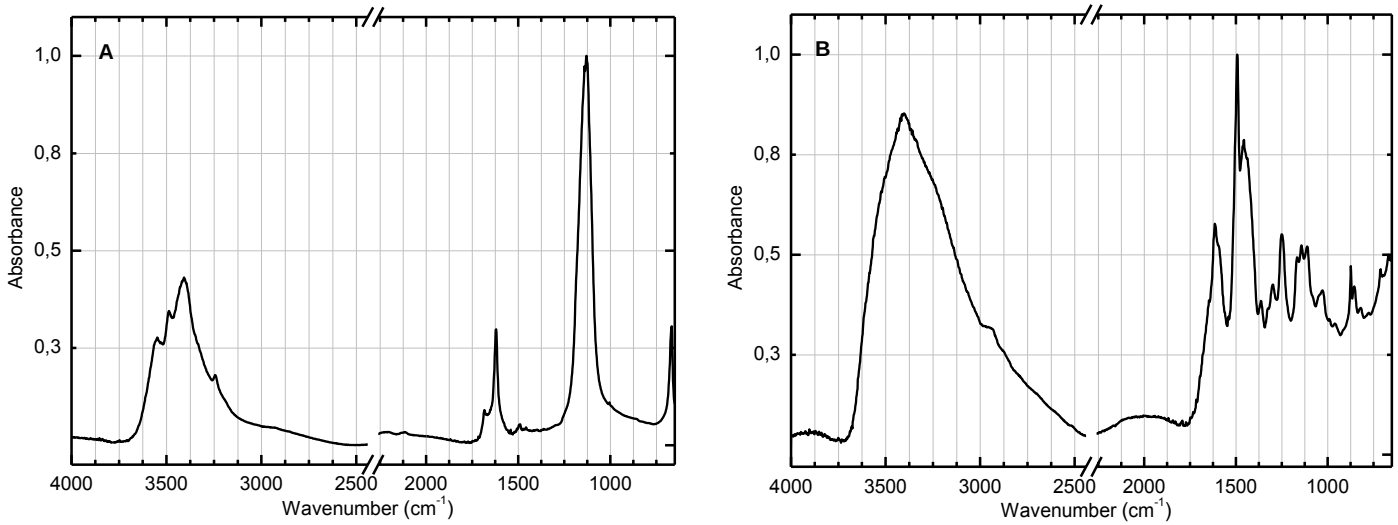


**Figure A24.** Infrared spectra of **A)** chapter 8, exp. 7 and **B)** chapter 9, exp. 2 pigment.

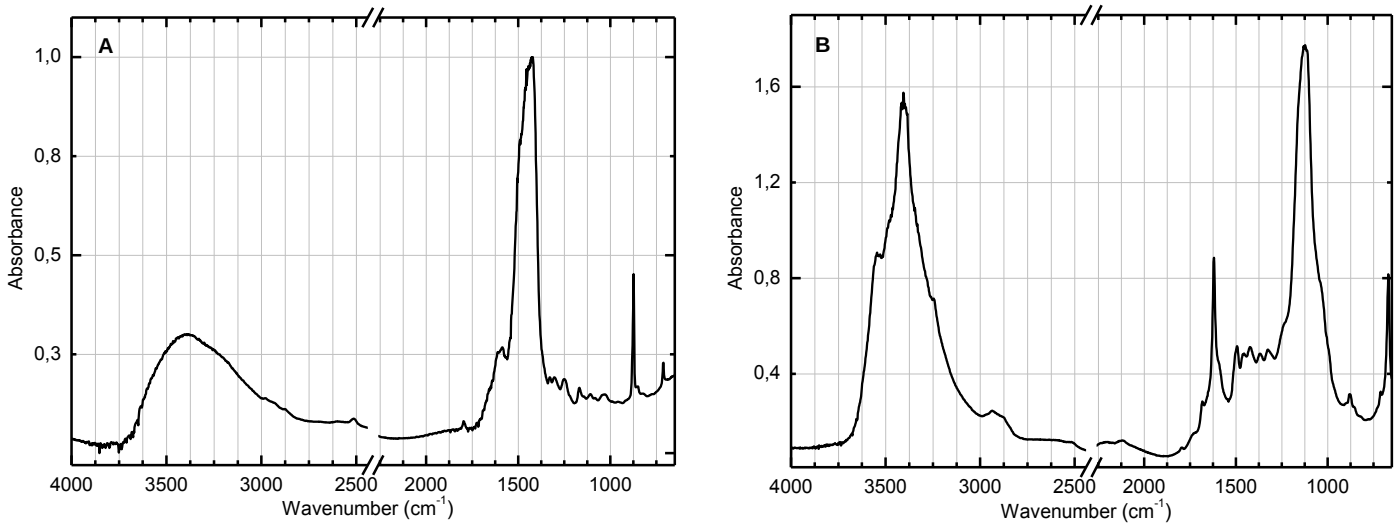


**Figure A25.** Infrared spectra of **A)** chapter 9, exp. 3 and **B)** chapter 9, exp. 5 pigment.

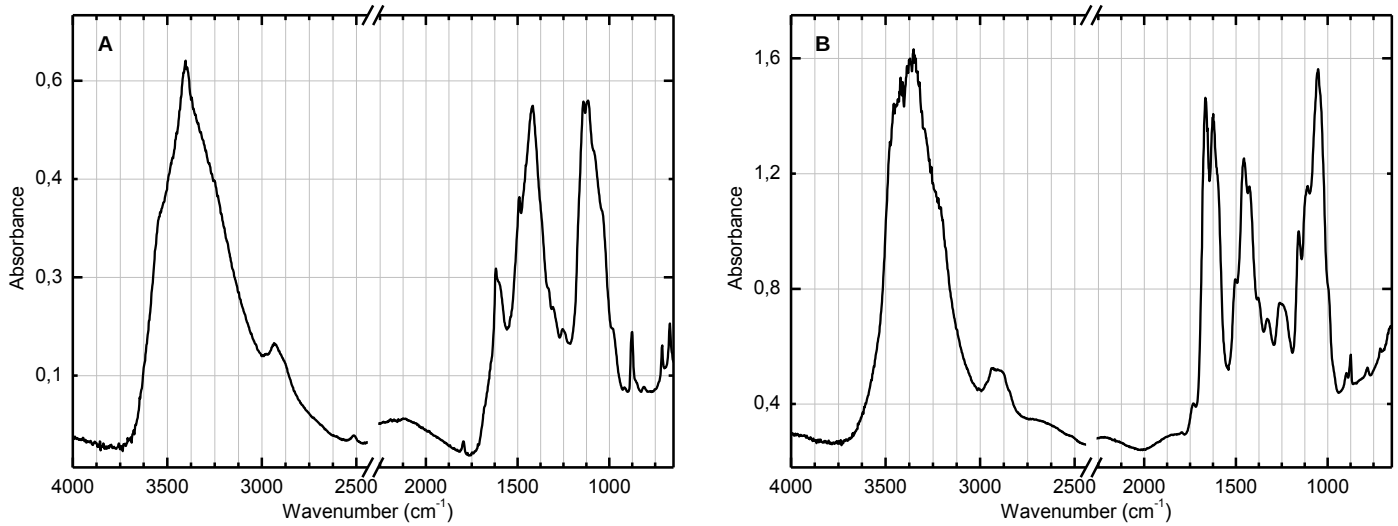


**Figure A26.** Infrared spectra of **A)** chapter 9, exp. 6 paint with gum-arabic and **B)** chapter 27, exp. 1 pigment.

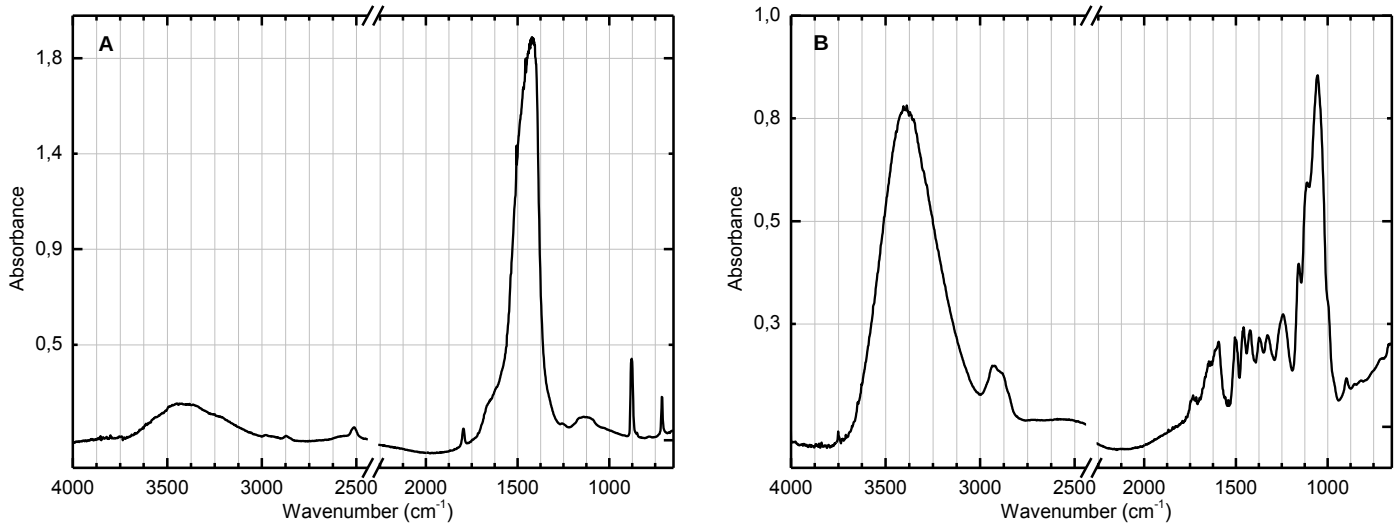


**Figure A27.** Infrared spectra of **A)** chapter 27, exp. 4 and **B)** chapter 44, exp. 1 pigment.

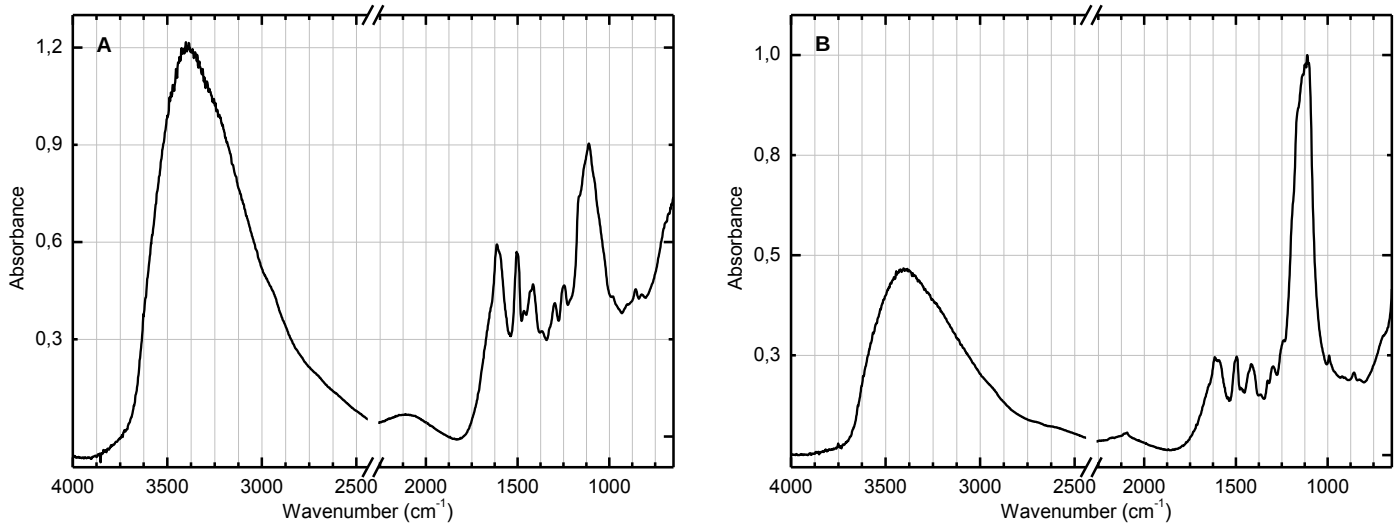


**Figure A28.** Infrared spectra of **A)** chapter 44, exp. 3 and **B)** chapter 44, exp. 4 pigment.

## 8.2. Infrared spectra of 19th century brazilwood lake pigments reconstructions

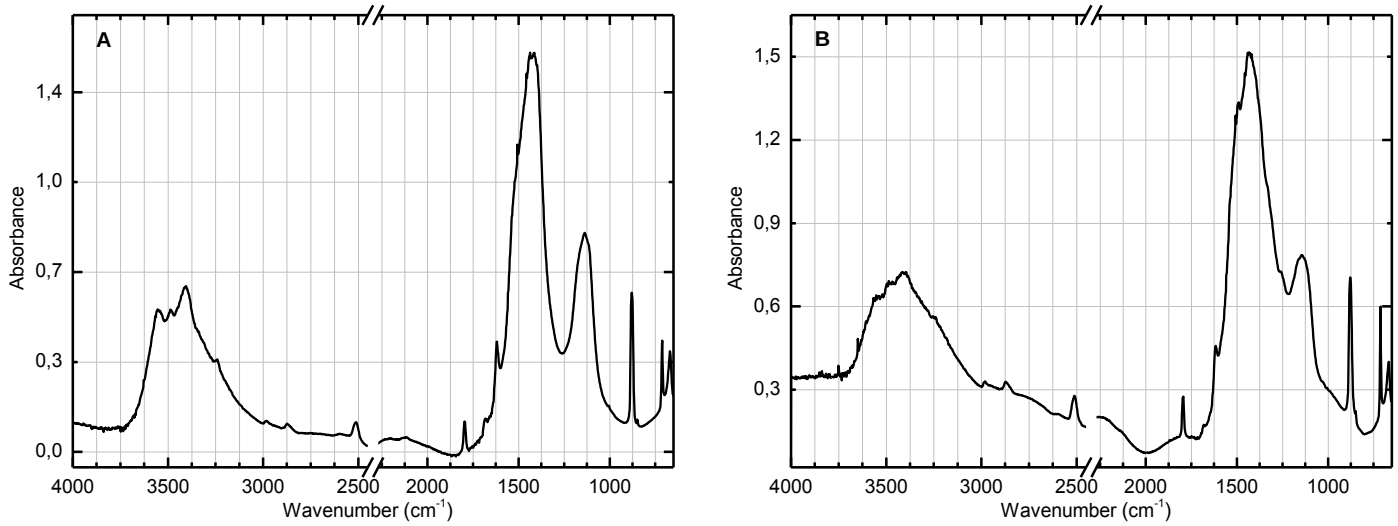


**Figure A29.** Infrared spectra of **A)** Rose Pink and **B)** Rose Pink II pigment.

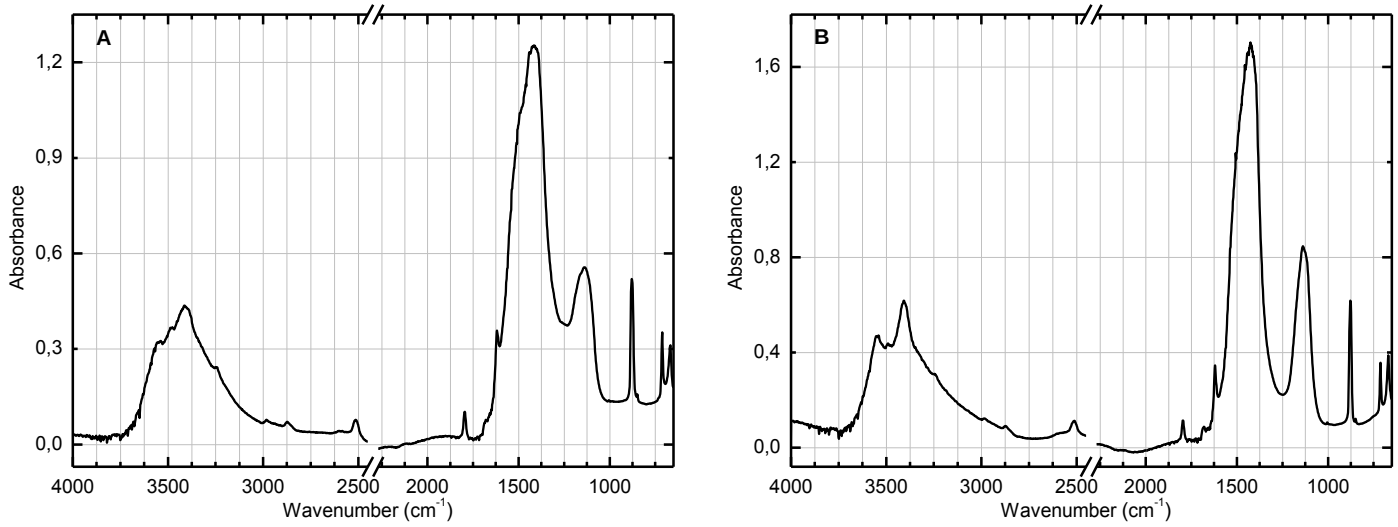


**Figure A30.** Infrared spectra of **A)** Rose Pink II (b) and **B)** Rose Pink Seacy's Formula pigment.

**APPENDIX 9.** FORS spectra of medieval brazilwood lake pigments reconstructions

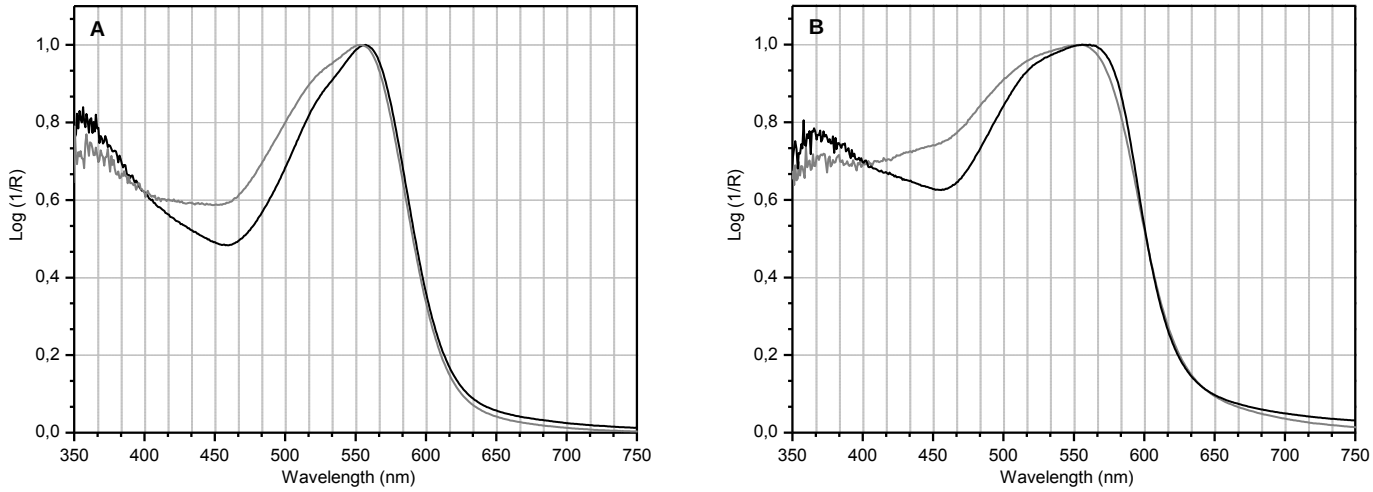


**Figure A31.** Absorbance spectra of chapter 8, exp. 6 painted with egg white (**—**) and parchment glue (—) on parchment applying **A)** few and **B)** many layers of paint.

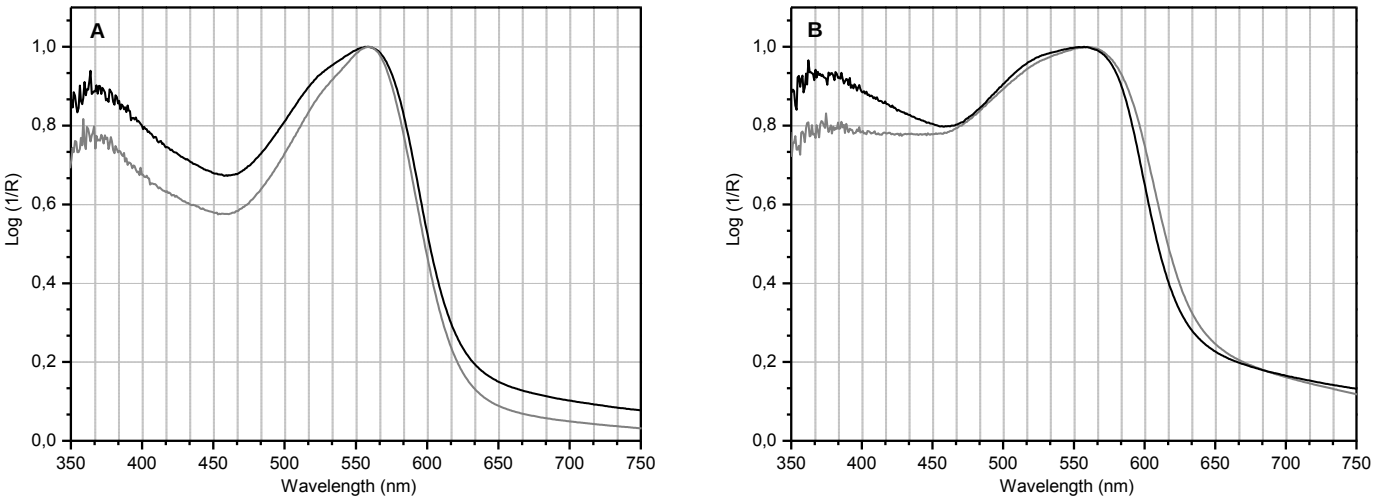


**Figure A32.** Absorbance spectra of chapter 9, exp. 5 painted with gum-arabic (**—**) and parchment glue (—) on parchment applying **A)** few and **B)** many layers of paint.

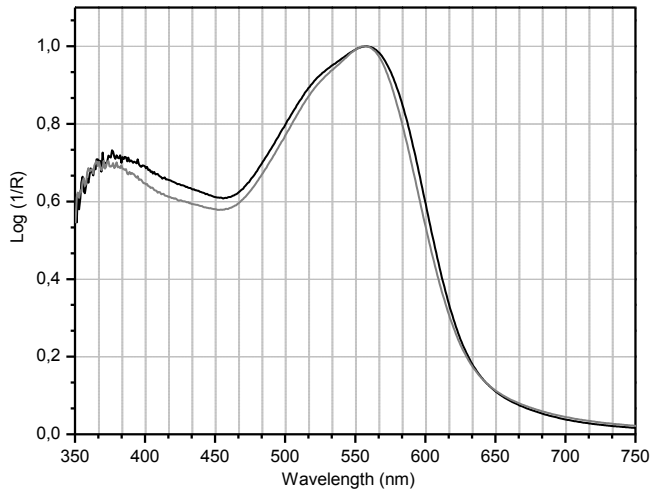


**Figure A33.** Absorbance spectra of chapter 27, exp. 4 painted with egg white (**—**) and parchment glue (—) on parchment applying few layers of paint.

**Appendix 10.** Fluorescence spectra of medieval brazilwood lake pigments reconstructions

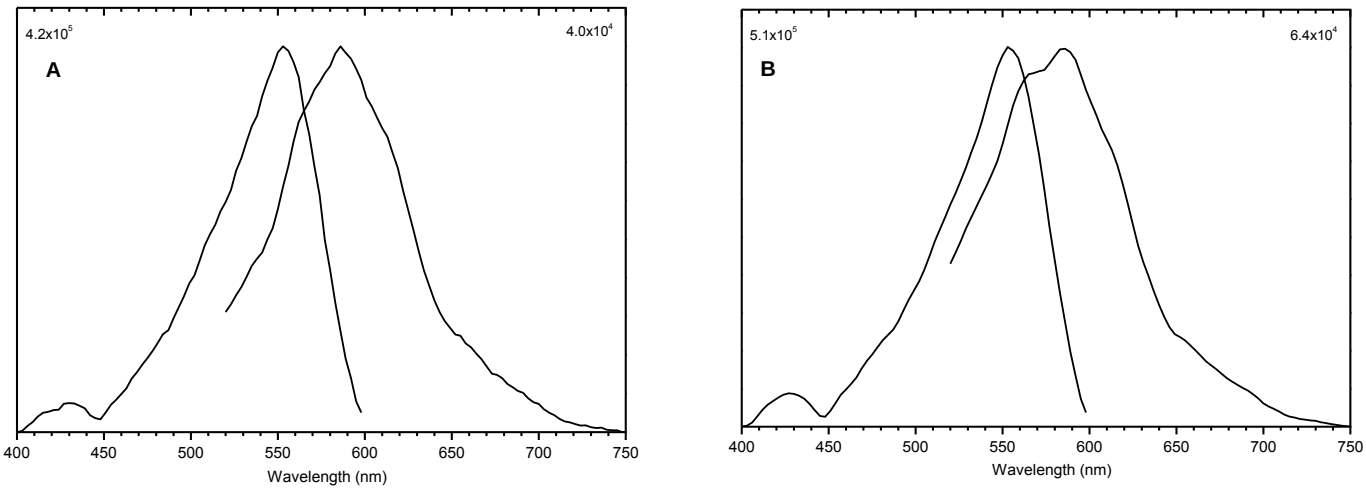


**Figure A34.** Emission and excitation spectra of **A)** chapter 8, exp. 3 and **B)** chapter 27, exp. 1 painted with gum-arabic on filter paper.

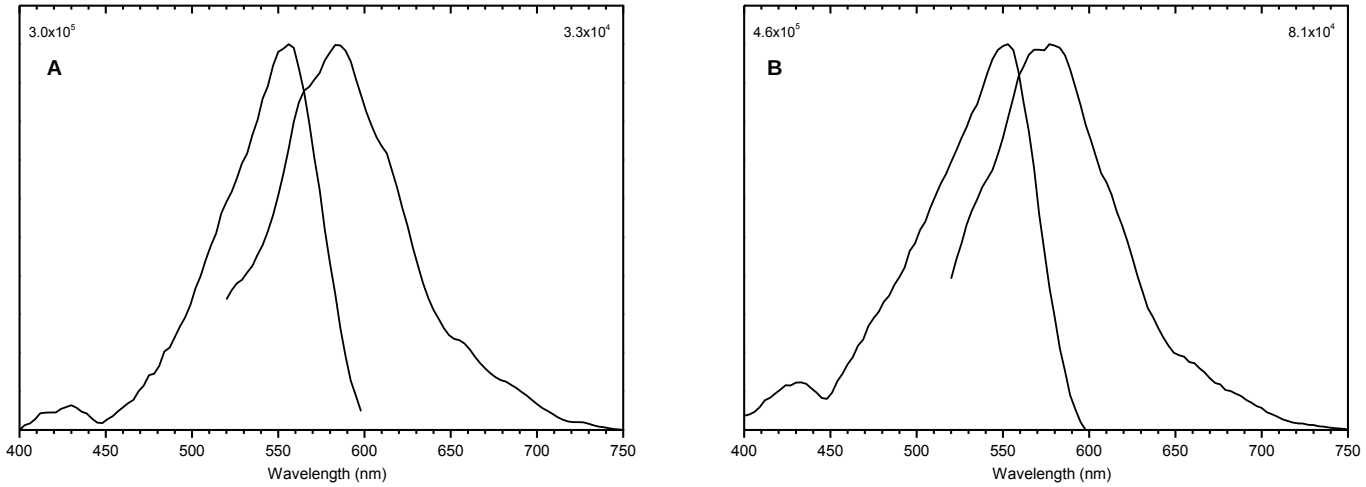


**Figure A35.** Emission and excitation spectra of **A)** chapter 27, exp. 4 and **B)** chapter 44, exp. 1 painted with gum-arabic on filter paper.

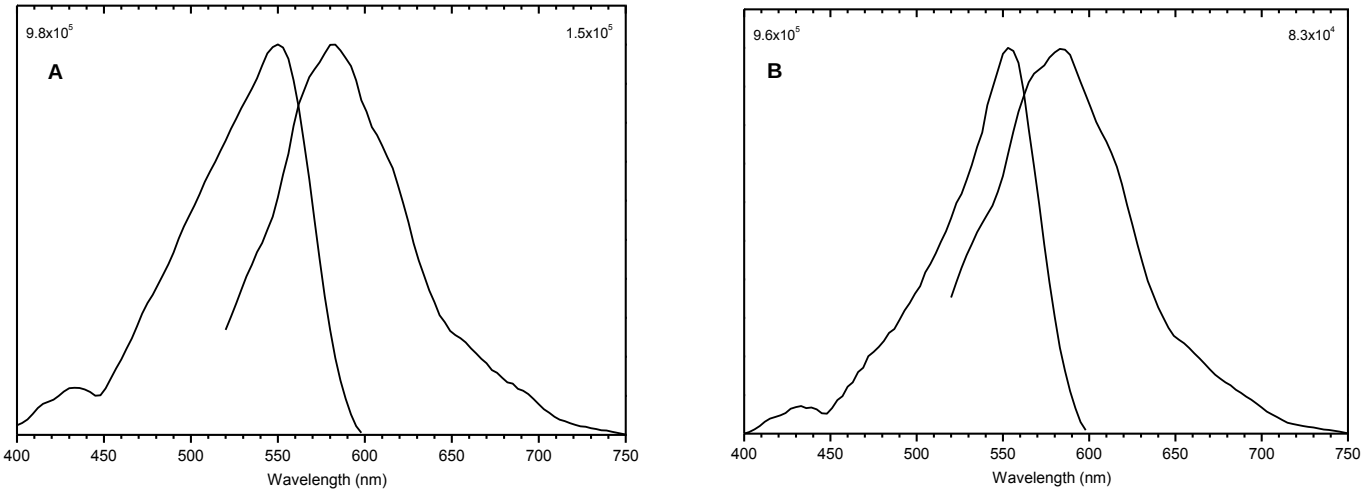


**Figure A36.** Emission and excitation spectra of **A)** chapter 44, exp. 3 and **B)** chapter 44, exp. 4 painted with gum-arabic on filter paper.

**Appendix 11.** Folios from the three French Books of Hours (15th century) from PNM

**Figure A37.** French books of hour from PNM: folio 22r (left) and folio 74r (right) from manuscript 22.

**Figure A38.** French books of hour from PNM: folio 76v from manuscript 22 (left) and folio 2r from manuscript 23 (right).

***Key:*** **(▲)** Infrared; **(◆)** SEM-EDS; **(■)** FORS; **(●)** μ-microspectrofluorimetry analysis.

**Figure A39.** French books of hour from PNM: folio 24r (left) and folio 46r (right) from manuscript 23.

**Figure A40.** French books of hour from PNM: folio 129r from manuscript 23 (left) and folio 17v from manuscript 24 (right).

***Key:*** **(▲)** Infrared; **(◆)** SEM-EDS; **(●)** µ-microspectrofluorimetry; **(★)** *In situ* µ-microspectrofluorimetry analysis.

**Figure A41.** French books of hour from PNM: folio 27r (left) and folio 53r (right) from manuscript 24.

**Figure A42.** French books of hour from PNM: folio 60r (left) and folio 116v (right) from manuscript 24.

***Key:*** **(▲)** Infrared; **(■)** FORS; **(●)** μ-microspectrofluorimetry; **(★)** *In situ* μ-microspectrofluorimetry analysis.

**Appendix 12.** Infrared spectra of pink and red colours μ-samples from case study

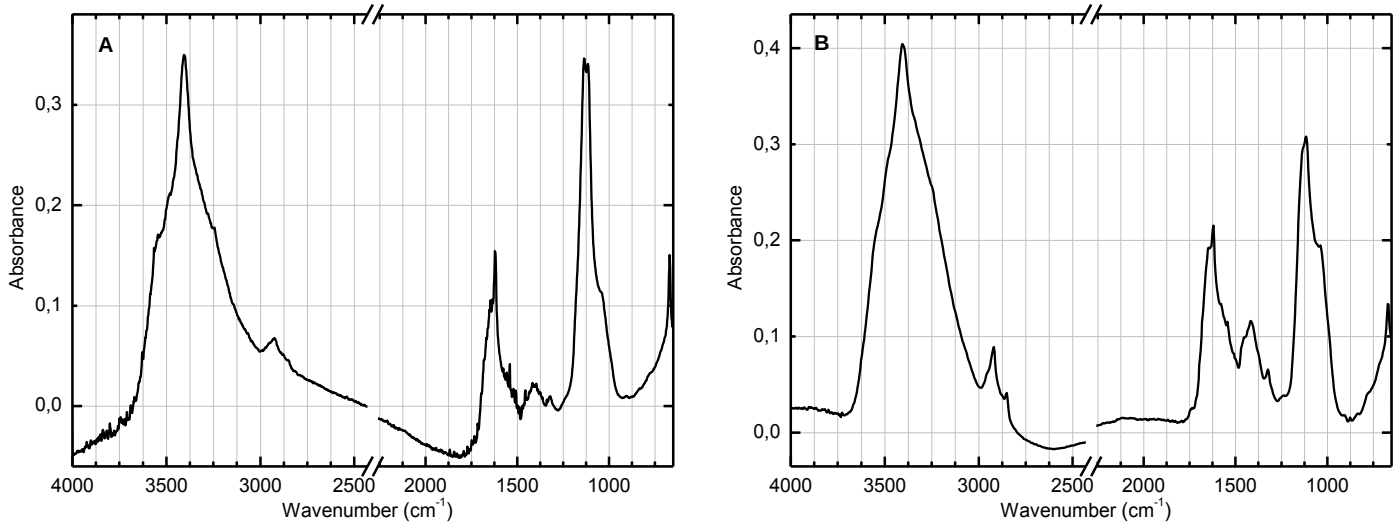


**Figure A43.** Infrared spectra of pink border μ-sample from **A)** folio 22r and **B)** folio 76v from manuscript 22.

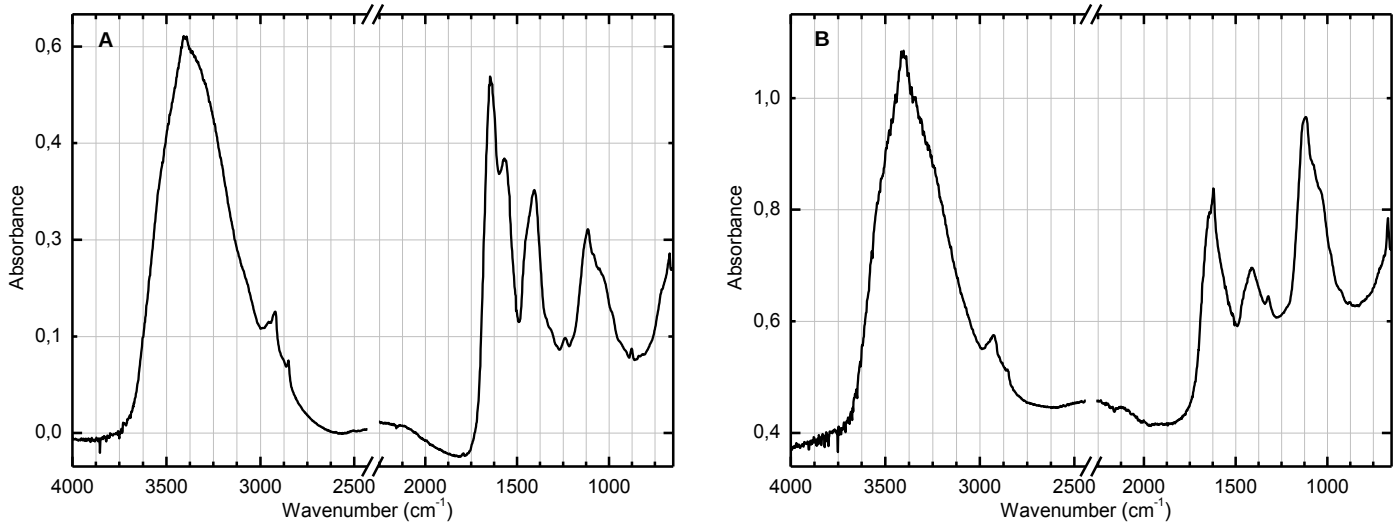


**Figure A44.** Infrared spectra of **A)** dark pink μ-sample and **B)** red varnish μ-sample from manuscript 23, folio 2r.

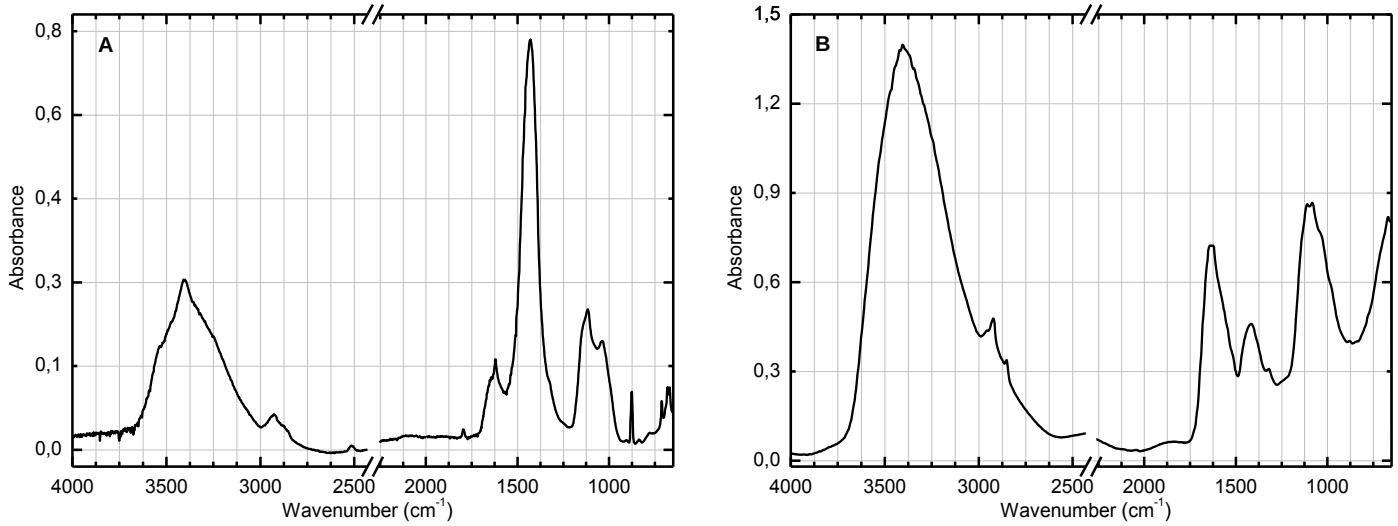


**Figure A45.** Infrared spectra of pink μ-sample from **A)** folio 24r and **B)** folio 46r from manuscript 23.

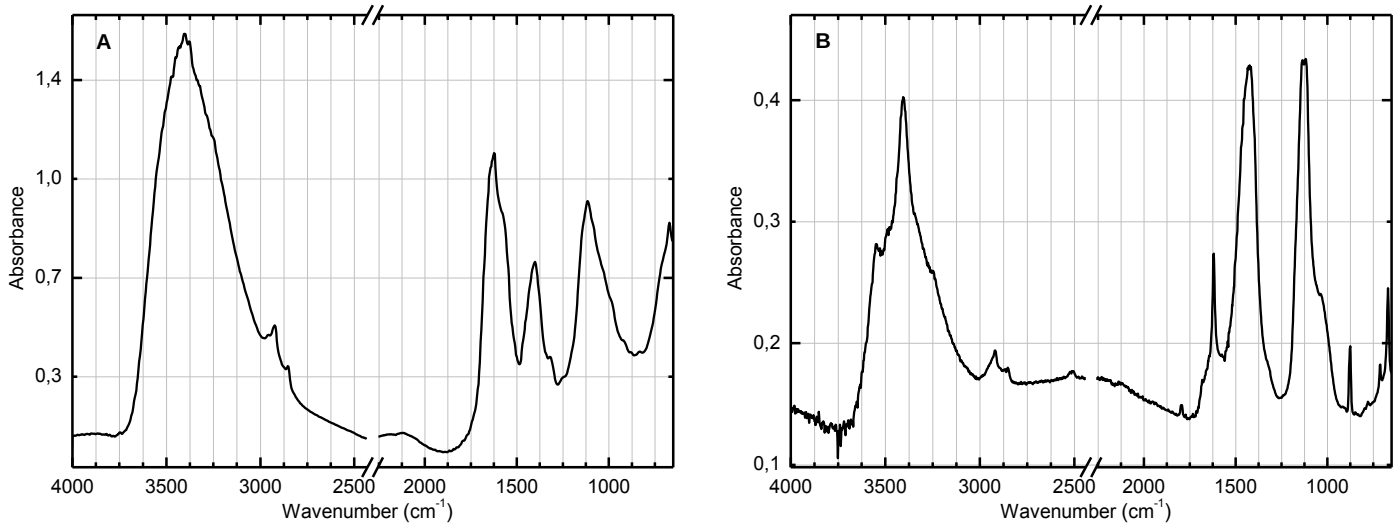


**Figure A46.** Infrared spectra of **A)** carmine µ-sample from manuscript 23, folio 129r and **B)** pink µ-sample from manuscript 24, folio 60r.

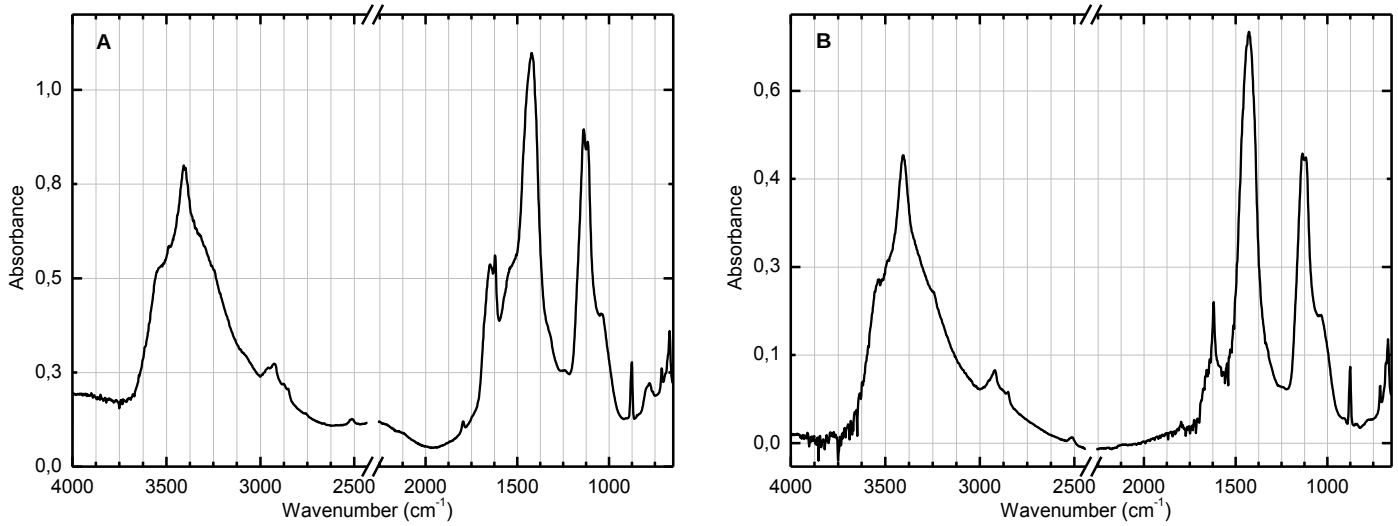


**Figure A47.** Infrared spectra of **A)** dark red µ-sample from folio 60r and **B)** pink µ-sample from folio 116v from manuscript 24.

**Appendix 13.** FORS spectra of pink and red colours from case study

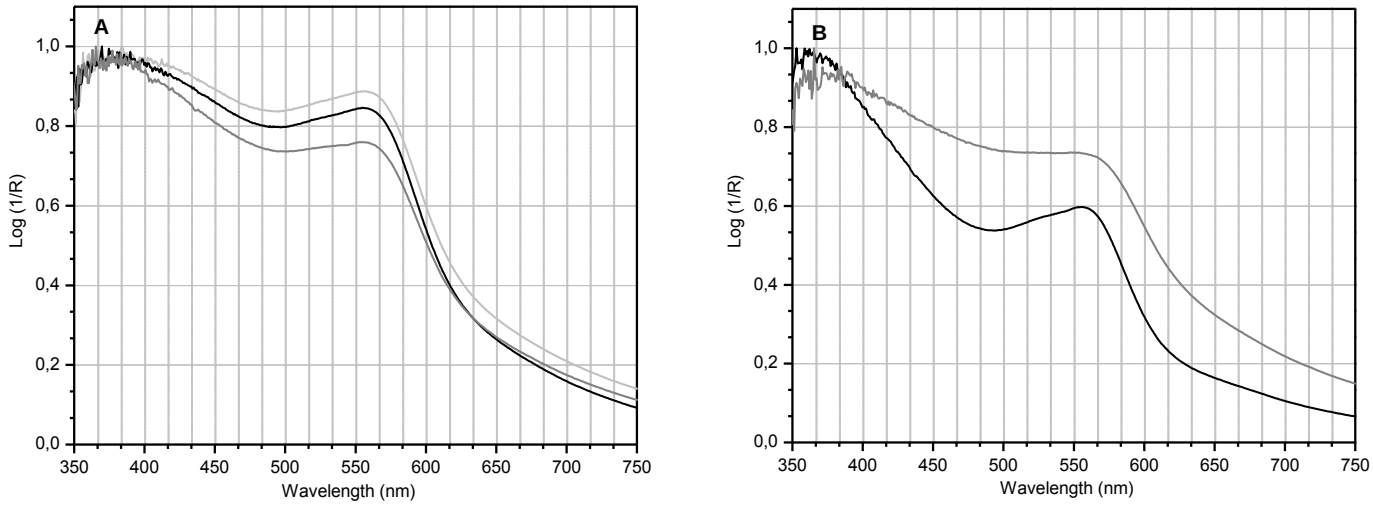


**Figure A48.** Absorbance spectra of **A)** carmine decorative borders and **B)** light pink paint from manuscript 22, folio 76v.

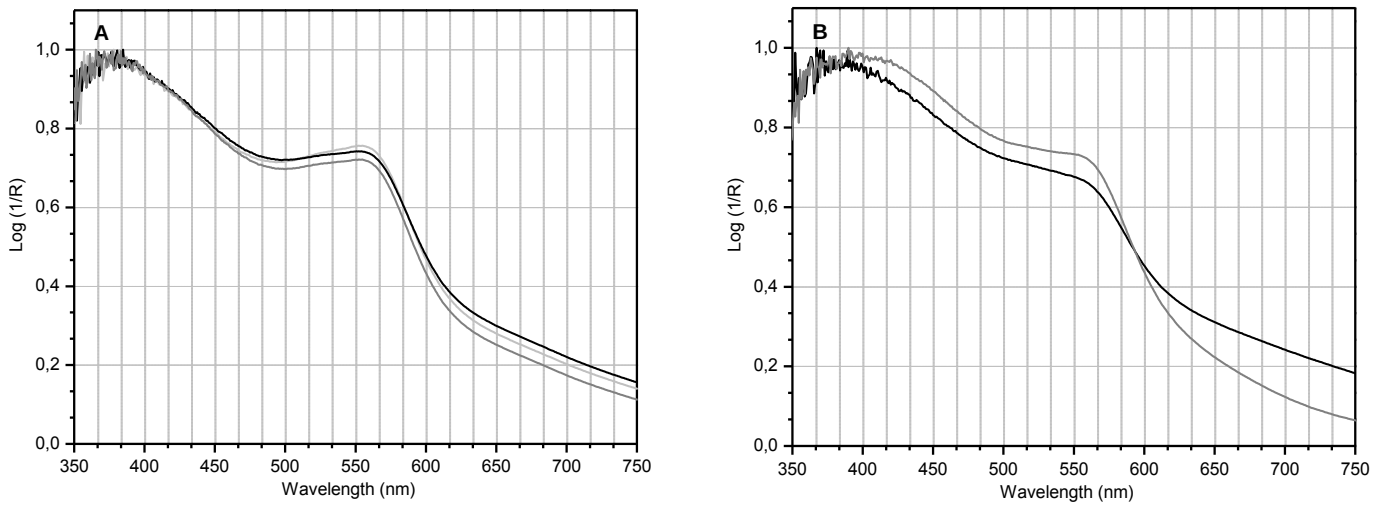


**Figure A49.** Absorbance spectra of **A)** dark pink paints from folio 76v and **B)** carmine decorative borders from folio 74r from manuscript 22.

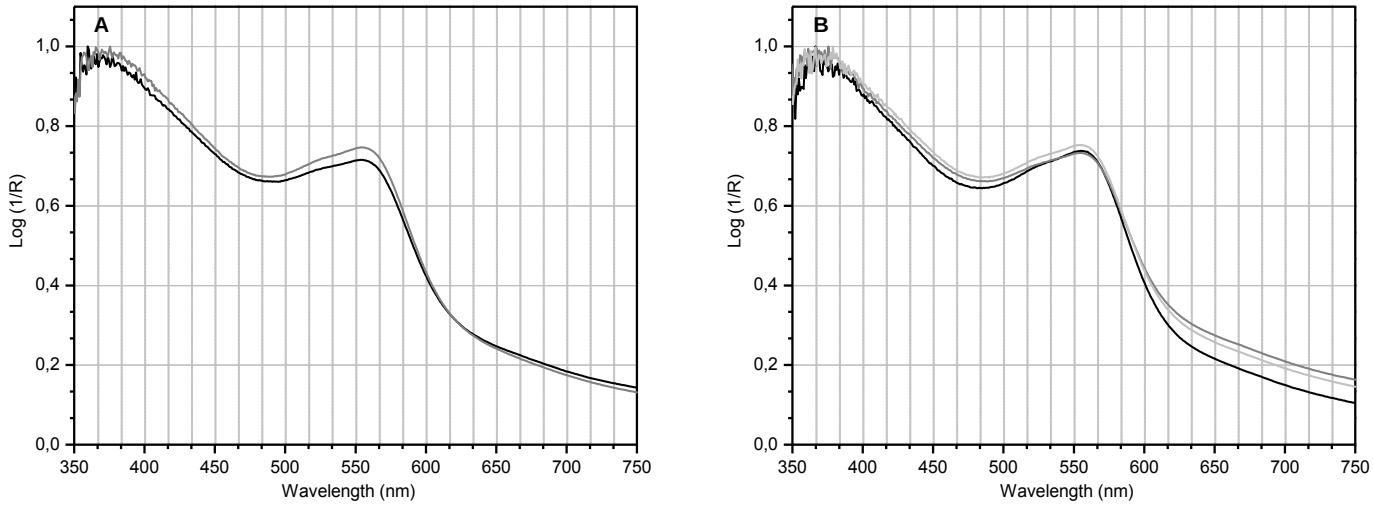


**Figure A50.** Absorbance spectra of carmine colours from **A)** folio 60r and **B)** folio 116v (from manuscript 24.

**Appendix 14.** Fluorescence spectra of pink and red colours from case study

### 14.2. µ-sample analysis

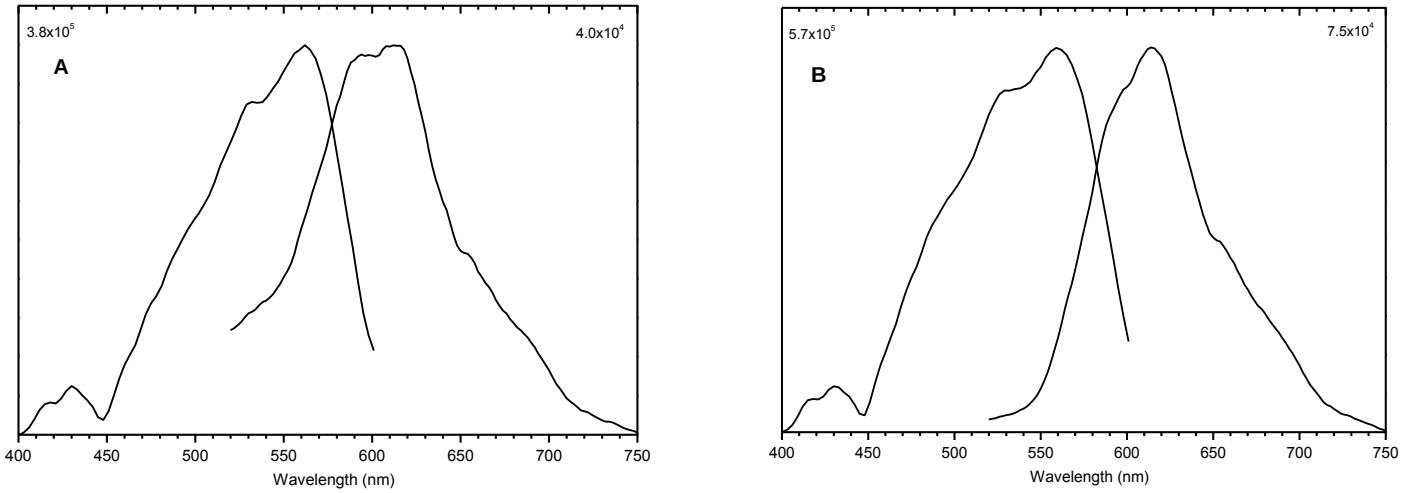


**Figure A51.** Emission and excitation spectra of **A)** dark-light pink µ-sample from folio 2r and **B)** pink µ-sample from folio 46r from manuscript 23.

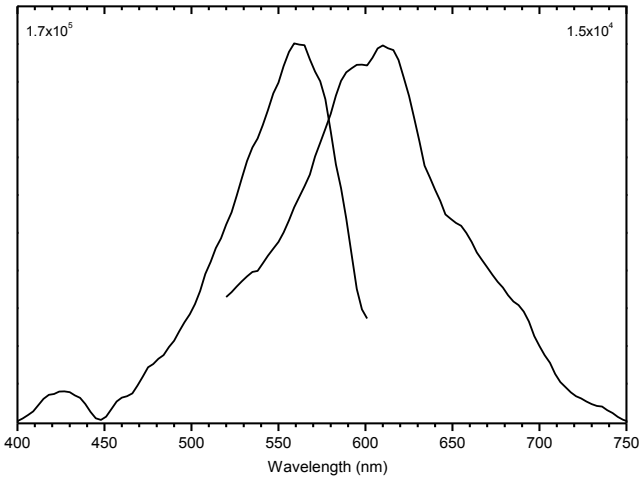


**Figure A52.** Emission and excitation spectra of carmine µ-sample from manuscript 23, folio 129r.

### 14.2. *In situ* analysis

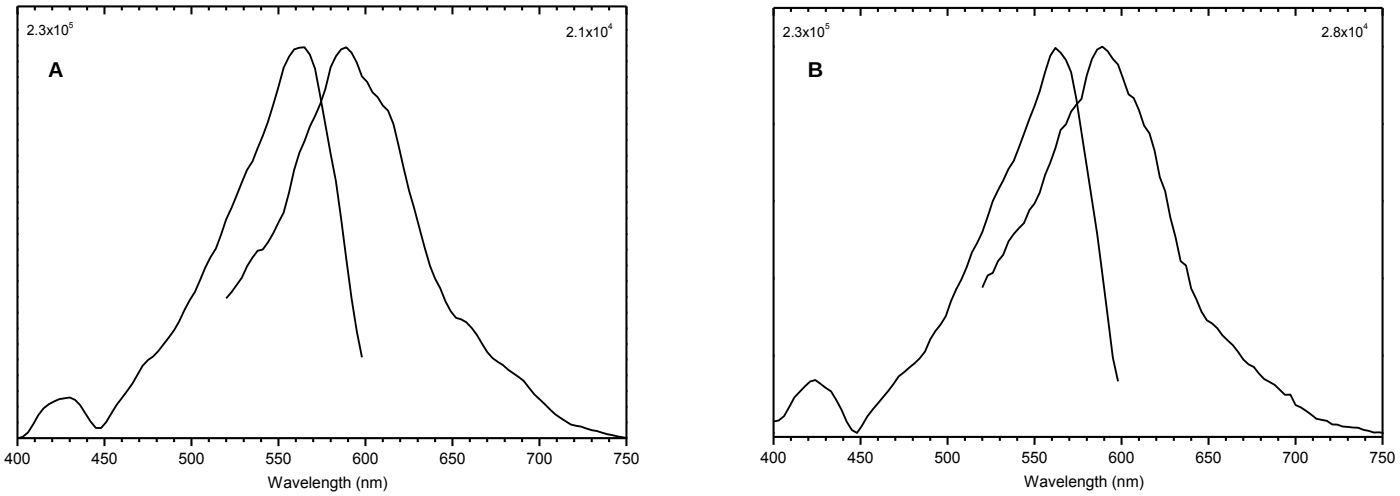


**Figure A53.** Emission and excitation spectra of **A)** red decoration and **B)** red decorative flower from manuscript 24, folio 116v.

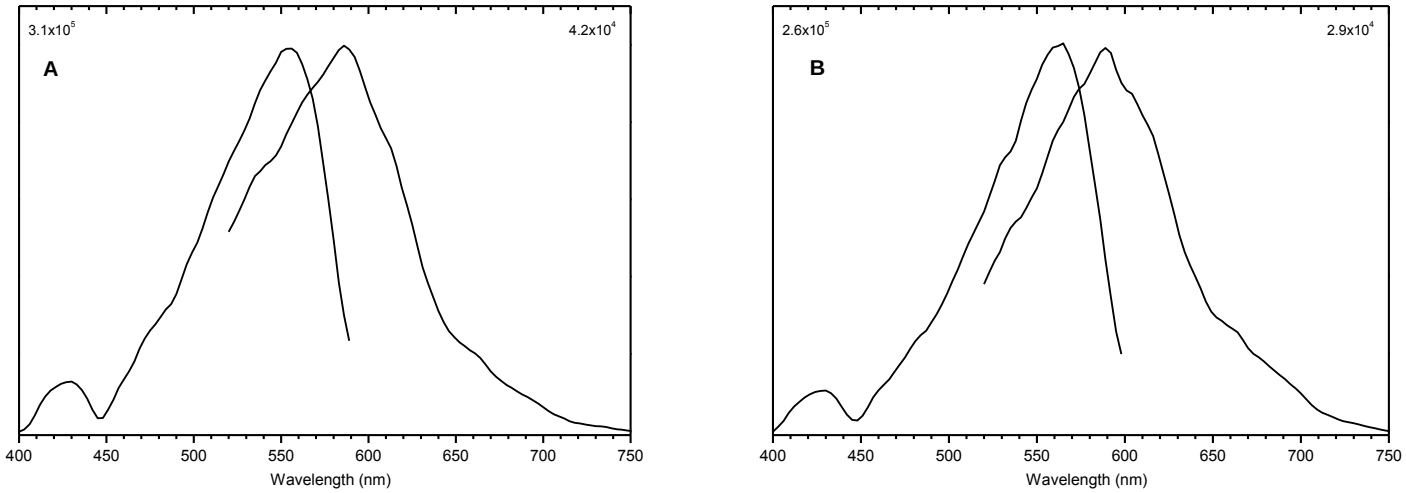


**Figure A54.** Emission and excitation spectra of **A)** pink young man's dress and **B)** red acanthus flower from manuscript 24, folio 116v.

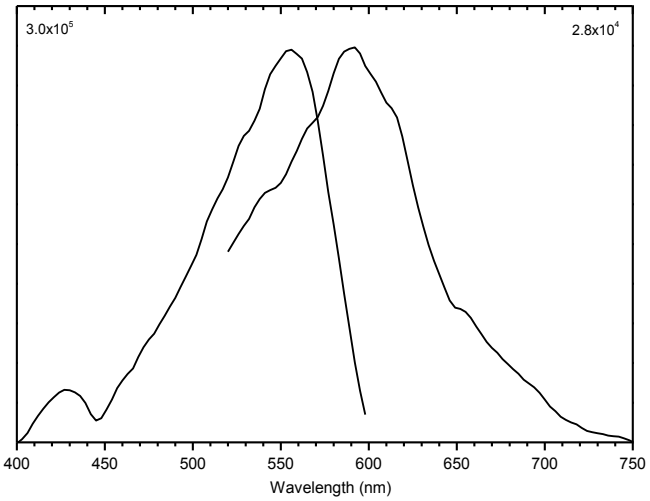


**Figure A55.** Emission and excitation spectra of pink man's dress from manuscript 24, folio 60r.